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PROCESOS BIOGEOQUÍMICOS EN UN ECOSISTEMA MARINO COSTERO DE INTERCAMBIO RESTRINGIDO. EL PUERTO DE MAHÓN.

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- Martínez-Soto, M. C., Basterretxea, G., Garcés, E., Anglès, S., Jordi, A., & Tovar-Sanchez, A. (2015). Species-specific variation in the phosphorus nutritional sources by microphytoplankton in a Mediterranean estuary. *Frontiers in Marine Science*, 2, 54.
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Resumen

Mahón es uno de los mayores puertos en el Mediterráneo Occidental. Emplazado en un clima típicamente mediterráneo está episódicamente perturbado por precipitaciones torrenciales y/o tormentas. Protegido de las corrientes costeras y con régimen micromareal, la renovación del agua en Mahón es generalmente baja y, en consecuencia, los impactos de las actividades humanas en su parte interior tienen consecuencias ambientales en todo el estuario. De forma característica, se genera un gradiente de nutrientes y metales entre la zona interior del puerto y el mar. Estas características han ofrecido una buena oportunidad para evaluar los diferentes procesos biogeoquímicos que allí ocurren y los factores que los modifican y/o controlan. En el presente trabajo se llevó a cabo una caracterización biogeoquímica de los dos principales compartimentos ambientales del ecosistema; los sedimentos y la columna de agua. También se caracterizaron y evaluaron las diferentes fuentes de nutrientes y/o metales (aguas subterráneas, torrentes, etc), y a través de enfoques experimentales se evalúo cómo afectan éstas a los procesos biogeoquímicos y en consecuencia al funcionamiento ecológico del ecosistema.

Los resultados de esta tesis revelan una secuencia de estados biogeoquímicos en el estuario de Mahón que resultan de la interacción entre la escorrentía, otros forzamientos no periódicos (vientos, las oscilaciones del nivel del mar) y las variaciones en la renovación del agua. Algunas variables y factores muestran una relación con la estacionalidad. Por ejemplo, la escorrentía se muestra como un motor importante de la circulación durante el invierno y de la afluencia de nutrientes. Por el contrario, durante el verano, la afluencia de agua dulce es despreciable y la renovación del agua se basa en impulsos de forzamiento atmosférico. A pesar de todas variaciones estacionales y/o episódicas, la concentración de los nutrientes inorgánicos muestra un gradiente persistente durante todo el año desde el interior hacia el exterior del estuario. El nitrógeno inorgánico disuelto y fosfato inorgánico presenta concentraciones elevadas en el interior del estuario durante casi todo el año. Esto refleja la importancia de los aportes terrestres en esta zona, sugiriendo la superposición de dos fuentes de nutrientes principales; una más permanente asociada a aportes antropogénicos y otra, más efímera, relacionada a eventos episódicos. De este modo se puede decir que las variaciones que ocurren entre las fuentes y los procesos biogeoquímicos internos determinan el balance de nutrientes del estuario.

En consecuencia, debido a las variaciones de las concentraciones de nutrientes y a los cambios en sus relaciones molares, la composición del fitoplancton y su productividad varía. Esto se ve reflejado en las variaciones espacio-temporales de la Chl a, que en

general muestra una marcada variación temporal, típica de este tipo de ecosistemas costeros impactados antrópicamente. Conocer estos cambios en la biomasa y composición del fitoplancton que co-existe en el estuario, ha permitido comprender los procesos que pueden estar regulando la producción primaria. Así, el fitoplancton en Mahón muestra una sucesión particular, dominada por la presencia de especies motiles durante todo el año, siendo los dinoflagelados y los cocolitoforidos los taxones predominantes durante todo el año. La evaluación de la actividad de la fosfatasa alcalina en el fitoplancton, sugiere la existencia de estrés por la poca disponibilidad de fosfato. Esta limitación ocurre tanto en verano como en otoño, a pesar de que las concentraciones de fosfato en el estuario son ligeramente superiores a la media del Mediterráneo y similares otras zonas costeras. Los bioensayos realizados en este trabajo muestran que la actividad de la fosfatasa alcalina persiste aun cuando concentraciones de fosfato en el medio son elevadas. Esos resultados sugieren que, en el caso de algunas especies de dinoflagelados, la actividad de esta enzima no está controlada por la concentración de fosfato en el medio y que en determinadas especies la fosfatasa alcalina podría indicar un uso selectivo de fósforo orgánico, o una respuesta metabólica lenta a los cambios en las formas de fósforo, en lugar de estrés fisiológico por la baja disponibilidad de fósforo.

Debido a los aportes de origen antropogénico que recibe, existe un deterioro en las condiciones ambientales en las que se encuentra el agua de Mahón. Esta pérdida de calidad no sólo se deriva de la elevada concentración de nutrientes. Además, existe un importante aporte de metales traza, que pueden jugar un papel importante en la co-limitación de la producción primaria. De aquí deriva la importancia de evaluar el contenido y la fuente de metales traza en las aguas superficiales en Mahón. Los resultados del presente trabajo muestran que la mayoría de los metales traza disueltos en las aguas del puerto exhiben, al igual que los nutrientes, un marcado gradiente de concentración (interior - exterior). En general, las concentraciones son más elevadas que en aguas abiertas. En particular, algunos metales como Cu, Fe y Pb presentan concentraciones especialmente altas en comparación con otras zonas costeras del Mediterráneo. Por otra parte, la concentración de algunos metales como el Cu y Zn, muestra un aumento durante el verano, coincidiendo con la temporada turística alta. El turismo masivo trae como consecuencia el aumento de la población y del tráfico marítimo, generando este último la resuspensión de los sedimentos superficiales en la parte interior del puerto. Al evaluar la concentración de metales en los sedimentos superficiales de Mahón, se observa que las concentraciones de Cu, Hg, Pb y Zn son mayores en comparación a otros lugares costeros del Mediterráneo.

La evaluación de los flujos de metales, mediante un balance de masas, ha permitido discernir cuál es la fuente más importe en Mahón. El resultado de este balance revela que, en comparación con otras fuentes putativas como la escorrentía, la deposición atmosférica y/o la descarga submarina de agua subterránea, los sedimentos son la principal fuente de los metales que se encuentran en la columna de agua. Esto sugiere la importancia de la resuspensión de los sedimentos producida por el tráfico de barcos de gran calado en el interior del puerto. El hecho de que varios de los metales que se encuentran en la columna de agua provengan de los sedimentos está sustentado por las buenas correlaciones entre las concentraciones de estos y los isotopos de Ra (^{223}Ra y ^{224}Ra) en las aguas superficiales del estuario. Mahón ha sido y sigue siendo un puerto comercial y turísticamente activo, con diversas actividades portuarias. La datación por isotopos de los núcleos de sedimentos obtenidos en el puerto, han proporcionado información sobre las variaciones en los metales desde el año 1890 hasta el presente. Este registro histórico muestra que el contenido de metales en el sedimento de Mahón es el resultado de las diversas actividades industriales desarrolladas en esta zona (textil, joyera, zapatería, electrónica, lácteos, la implantación de centrales termoeléctricas, la utilización de la gasolina con plomo, entre otras). A partir de 1978, la construcción de un sistema de recogida de aguas urbanas y su vertido mediante un emisario con salida al mar, fuera del puerto, permitió la reducción de la concentración de metales en las aguas del puerto. Aunado a esto, los resultados del factor de enriquecimiento (EF) y del índice de geoacumulación (Igeo) señalan que los sedimentos profundos de Mahón están enriquecidos en algunos metales y sus concentraciones se han ido estabilizando hacia los años más recientes. Esto sugiere que cualquier alteración física significativa del ambiente sedimentario puede provocar la resuspensión de cantidades significativas de algunos metales traza. En general, los resultados de esta tesis aportan gran información para la comprensión del funcionamiento de este tipo de ecosistemas y dejan en evidencia la particularidad e importancia de los procesos biogeoquímicos que allí se desarrollan.

Resum

Mahón és un dels majors ports en el Mediterrani Occidental. Emplaçat en un clima típicament mediterrani està episòdicament pertorbat per precipitacions torrencials i/o tempestes. Protegit dels corrents costaners i amb règim micromareal, la renovació de l'aigua a Mahón és generalment baixa i, en conseqüència, els impactes de les activitats humanes en la seva part interior tenen conseqüències ambientals en tot l'estuari. De forma característica, es genera un gradient de nutrients i metalls entre la zona interior del port i el mar. Aquestes característiques han ofert una bona oportunitat per avaluar els diferents processos biogeoquímics que allí ocorren i els factors que els modifiquen i/o controlen. En el present treball, es va dur a terme una caracterització biogeoquímica dels dos principals compartiments ambientals de l'ecosistema; els sediments i la columna d'aigua. També es van caracteritzar i avaluar les diferents fonts de nutrients i/o metalls (aigües subterrànies, torrents, etc), i a través d'enfocaments experimentals es va avaluar com afecten aquestes als processos biogeoquímics i en conseqüència al funcionament ecològic de l'ecosistema.

Els resultats d'aquesta tesi revelen una seqüència d'estats biogeoquímics en l'estuari de Maó que resulten de la interacció entre el vessament, altres forçaments no periòdics (vents, les oscil·lacions del nivell del mar) i les variacions en la renovació de l'aigua. Algunes variables i factors mostren una relació amb l'estacionalitat. Per exemple, el vessament es mostra com un motor important de la circulació durant l'hivern i de l'afluència de nutrients. Per contra, durant l'estiu, l'afluència d'aigua dolça és menyspreable i la renovació de l'aigua es basa en impulsos forçament atmosfèrics. Malgrat totes les variacions estacionals i/o episòdiques, la concentració dels nutrients inorgànics mostra un gradient persistent durant tot l'any des de l'interior cap a l'exterior de l'estuari. El nitrogen inorgànic dissolt i el fosfat inorgànic presenten concentracions elevades a l'interior de l'estuari durant gairebé tot l'any. Això reflecteix la importància de les aportacions terrestres en aquesta zona, suggerint la superposició de dues fonts de nutrients principals; una de més permanent associada amb aportacions antropogènics i una altra, de més efímera, relacionada a esdeveniments episòdics. D'aquesta manera es pot dir que les variacions que ocorren entre les fonts i els processos biogeoquímics interns determinen el balanç de nutrients de l'estuari.

En conseqüència, a causa de les variacions de les concentracions de nutrients i als canvis en les seves relacions molars, la composició del fitoplàncton i la seva productivitat varia. Això es veu reflectit en les variacions espaciotemporals de la Chl a, que en general mostra una marcada variació temporal, típica d'aquest tipus

d'ecosistemes costaners impactats antròpicament. Conèixer aquests canvis en la biomassa i composició del fitoplàncton que coexisteix en l'estuari, ha permès comprendre els processos que poden estar regulant la producció primària. Així, el fitoplàncton de Maó mostra una successió fitoplanctònica particular, dominada per la presència d'espècies mótiles durant tot l'any, sent els dinoflagel·lats i els coccolitoforis els taxons predominants durant tot l'any. L'avaluació de l'activitat de la fosfatasa alcalina en el fitoplàncton, suggereix l'existència d'estrès per la poca disponibilitat de fosfat. Aquesta limitació ocorre tant a l'estiu com a la tardor, a pesar que les concentracions de fosfat en l'estuari són lleugerament superiors a la mitjana del Mediterrani i similars a les d'altres zones costaneres. Els bioassaigs realitzats en aquest treball mostren que l'activitat de la fosfatasa alcalina persisteix tot i que concentracions de fosfat en el mitjà són elevades. Aquests resultats suggereixen que, en el cas d'algunes espècies de dinoflagel·lats, l'activitat d'aquest enzim no està controlada per la concentració de fosfat en el mitjà, i que en determinades espècies la fosfatasa alcalina podria indicar un ús selectiu de fòsfor orgànic, o una resposta metabòlica lenta als canvis en les formes de fòsfor, en lloc d'estrès fisiològic per la baixa disponibilitat de fòsfor.

A causa de les aportacions d'origen antropogènic que rep, existeix una deterioració en les condicions ambientals en les quals es troba l'aigua de Maó. Aquesta pèrdua de qualitat no només es deriva de l'elevada concentració de nutrients, sinó que a més existeix una important aportació de metalls traça que poden jugar un paper important en la colimitació de la producció primària. D'aquí deriva la importància d'avaluar el contingut i la font de metalls traça en les aigües superficials a Maó. Els resultats del present treball mostren que la majoria dels metalls traça dissolts en les aigües del port exhibeixen, igual que els nutrients, un marcat gradient de concentració (interior - exterior). En general, les concentracions de metalls en les aigües del port són més elevades que en aigües obertes. En particular, alguns metalls com a Cu, Fe i Pb hi presenten concentracions especialment altes en comparació amb altres zones costaneres del Mediterrani. D'altra banda, la concentració d'alguns metalls com el Cu i Zn, hi mostra un augment durant l'estiu, coincidint amb la temporada turística alta. El turisme massiu porta com a conseqüència l'augment de la població i del tràfic marítim, generant aquest últim la resuspensió dels sediments superficials en la part interior del port. En avaluar la concentració de metalls en els sediments superficials de Maó, s'hi observa que les concentracions d'alguns metalls com a Cu, Hg, Pb i Zn són majors en comparació amb altres llocs costaners del Mediterrani.

L'avaluació dels fluxos de metalls, mitjançant un balanç de masses, ha permès destriar quin és la font més import a Maó. El resultat d'aquest balanç revela que, en comparació amb altres fonts putatives com el vessament, la deposició atmosfèrica i/o la descàrrega submarina d'aigua subterrània, els sediments són la principal font dels metalls que es troben en la columna d'aigua. Això suggereix la importància de la resuspensió dels sediments produïda pel tràfic de vaixells de gran importància a l'interior del port. El fet que diversos metalls que es troben en la columna d'aigua provinguin dels sediments, està sustentat per les bones correlacions entre les concentracions dels metalls i els isòtops de Ra (^{223}Ra i ^{224}Ra) en les aigües superficials de l'estuari. Maó ha estat i segueix sent un port comercial i turísticament actiu, amb diverses activitats portuàries. La datació per isòtops dels nuclis de sediments obtinguts en el port, ha proporcionat informació sobre les variacions en els metalls des de l'any 1890 fins al present. Aquest registre històric mostra que el contingut de metalls en el sediment de Maó és el resultat de les diverses activitats industrials desenvolupades en aquesta zona (tèxtil, joiera, sabateria, electrònica, làctics, la implantació de centrals termoelèctriques, la utilització de la gasolina amb plom, entre altres). A partir de 1978, la construcció d'un sistema de recollida d'aigües urbanes i el seu abocament mitjançant un emissari amb sortida al mar, fora del port, va permetre la reducció de la concentració de metalls en les aigües del port. Juntament amb això, els resultats del factor d'enriquiment (EF) i de l'índex de geoacumulació (Igeo) assenyalen que els sediments profunds de Maó estan enriquits en alguns metalls i les seves concentracions s'han anat estabilitzant cap als anys més recents. Això suggereix que qualsevol alteració física significativa de l'ambient sedimentari pot provocar la resuspensió de quantitats significatives d'alguns metalls traça. En general, els resultats d'aquesta tesi aporten gran informació per a la comprensió del funcionament d'aquest tipus d'ecosistemes i deixen en evidència la particularitat i importància dels processos biogeoquímics que allà es desenvolupen.

Summary

Mahon is one of the largest ports in the Western Mediterranean. Located in a typically Mediterranean climate is inconsistently disturbed by torrential rains and/or storms. As it is protected by coastal currents and has a microtidal regime, the renewal of water in Mahon is generally low and, accordingly, the impacts of human activities on its inner part have environmental consequences throughout the estuary. A characteristic gradient of nutrients and metals between the inner harbor area and the sea is generated. These features have provided a good opportunity to evaluate the different biogeochemical processes that are taking place there and the factors that modify and/or control them. In the present work, we carried out a biogeochemical characterization of the two main environmental compartments of the ecosystem; the sediment and the water column. The different sources of nutrients and/or metals (groundwater, torrents, etc) were also evaluated and characterized, and, by means of experimental approaches, it was assessed how these different sources affected the biogeochemical processes and, consequently, the ecological functioning of the ecosystem.

The results of this thesis reveal a sequence of biogeochemical states in the estuary of Mahon that result from the interaction between the runoff, other non-recurrent forcings (winds, the oscillations of the sea level), and the variations in water renewal. Some variables and factors show a relationship with the seasonality. For example, the runoff is shown to be an important engine of the movement during the winter and nutrient influx. On the contrary, during the summer, the influx of freshwater is negligible and the renewal of water is based on atmospheric forcing impulses. Despite all seasonal and/or episodic variations, the concentration of inorganic nutrients shows a persistent gradient throughout the year from the inside toward the outside of the estuary. The dissolved inorganic nitrogen and inorganic phosphate present high concentrations in the interior of the estuary during almost the entire year. This reflects the importance of the land contributions in this area, suggesting the overlap of two sources of major nutrients; a more permanent one associated with anthropogenic inputs and another, more ephemeral, related to episodic events. Therefore, we can say that the variations that occur between the sources and internal biogeochemical processes determine the nutrient balance of the estuary.

Accordingly, due to variations in nutrient concentrations and changes in their molar relationships, phytoplankton composition and productivity vary. This is reflected in the space-temporal variations of chl a, which in general show a marked seasonal variation, typical of this type of anthropically-impacted coastal ecosystems. The

comprehension of these changes in biomass and composition of the phytoplankton that co-exist in the estuary, has allowed us to understand the processes that regulate primary production in it. Thus, the phytoplankton of Mahon shows a particular phytoplankton succession, dominated by the presence of motile species during the whole year, being the dinoflagellates and the coccolithphorids the predominant taxa throughout the year. The evaluation of the activity of alkaline phosphatase in the phytoplankton, suggests the existence of stress due to the poor availability of phosphate. This limitation occurs both in the summer and the autumn, despite that phosphate concentrations in the estuary are slightly higher than the average for the Mediterranean and like other coastal areas. The Bioassays performed in this paper show that the activity of the alkaline phosphatase persists even when phosphate concentrations in the environment are high. These results suggest that, in the case of some species of dinoflagellates, the activity of this enzyme is not controlled by the phosphate concentration in the environment. Besides, the results suggest that in certain species the alkaline phosphatase could indicate a selective use of organic phosphorus, or a slow metabolic response to changes in the phosphorus forms instead of physiological stress by the low availability of phosphorus.

Due to the contributions of anthropogenic origin that the estuary receives, the environmental conditions of water in Mahon are deteriorating. This loss of quality derives not only from the high concentration of nutrients, but there is also an important contribution of trace metals, which can play an important role in the co-limitation of primary production. Hence it is of high importance to assess the content and the source of trace metals in surface waters in Mahon. The results of this work show that the majority of the trace metals dissolved in the waters of the port exhibit, like the nutrients, a marked concentration gradient (inside - outside). In general, metal concentrations in the port are higher than in open waters. In particular, some metals as Cu, Fe and Pb show especially high concentrations in comparison to other coastal areas of the Mediterranean. On the other hand, the concentration of some metals such as Cu and Zn increase during the summer, coinciding with the high tourist season. Mass tourism brings as a consequence the increase of population and maritime traffic, generating the latter resuspension of surface sediments in the inner part of the harbor. When metal concentration in the superficial sediments of Mahon was evaluated, it was noted that the concentrations of some metals such as Cu, Hg, Pb and Zn were higher in comparison to other coastal places of the Mediterranean.

The evaluation of metal flows, by means of a mass balance, has allowed discerning what the main source in Mahon is. The result of this evaluation reveals that, in comparison with other putative sources as the runoff, atmospheric deposition and/or submarine discharge of underground water, sediments are the main source of the metals found in the water column. This highlights the importance of resuspension of sediment produced by the traffic of ships with deep drafts in the interior of the port. The fact that several of the metals that were located in the water column come from sediments was supported by good correlations between metal concentrations and Ra isotopes (223Ra and 224Ra) in the surface waters of the estuary. Mahon has been and still is a commercial and touristically active port, with various port activities. Isotopes dating of sediment cores obtained in the port, have provided information on the changes in the metals from 1890 until the present. This historical record shows that metal content in the sediment of Mahon is the result of the various industrial activities developed in this area (textiles, jewelry, shoemaking, electronics, dairy products, the introduction of thermoelectric plants, the use of leaded gasoline, among others). Since 1978, the construction of a system for the collection of urban waters and their discharge by an emissary with exit to the sea, outside the port, led to the reduction in metal concentration in the waters of the port. Coupled with this, the results of the enrichment factor (EF) and the geoaccumulation index (Igeo) indicate that deep sediments in Mahon are enriched in some metals and that their concentrations have stabilized during the most recent years. This suggests that any significant physical alteration of the sedimentary environment can cause resuspension of significant quantities of some trace metals. In general, the results of this thesis provide great information for the understanding of the functioning of this type of ecosystem and evidence the particularity and importance of the biogeochemical processes that take place there.

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Introducción General

Mediterráneo y sus características biogeoquímicas

Hidrografía

El Mediterráneo es un mar semi-cerrado, con escaso intercambio de agua y rodeado por zonas altamente pobladas (Fig. 1). La elevada presión antropogénica y la baja renovación de sus aguas lo hacen vulnerable a los cambios tanto de origen natural como antropogénicos. De hecho, con aproximadamente 3.860 km de longitud y una profundidad media de 1,5 km, el Mediterráneo es el mar europeo semi-cerrado de mayor tamaño y el segundo mar interior más grande del mundo (Astraldi et al., 2002; Rodrıguez, 1982). La cuenca mediterránea se extiende por más de 2,5 millones de km^2 conformados por distintas sub-cuencas conectadas entre sí mediante estrechos (Astraldi et al., 1999; Candela et al., 1999). Así, el estrecho de Sicilia actúa como barrera geografía e hidrológica separando dos grandes subcuencas, con características bien diferenciadas. La cuenca occidental presenta una mayor influencia Atlántica ya que comunica con este océano a través del Mar de Alborán y estrecho de Gibraltar. La cuenca oriental, conecta con el mar Negro por los estrechos del Bósforo y de los Dardanelos y recibe la influencia del mar Rojo a través del canal de Suez.

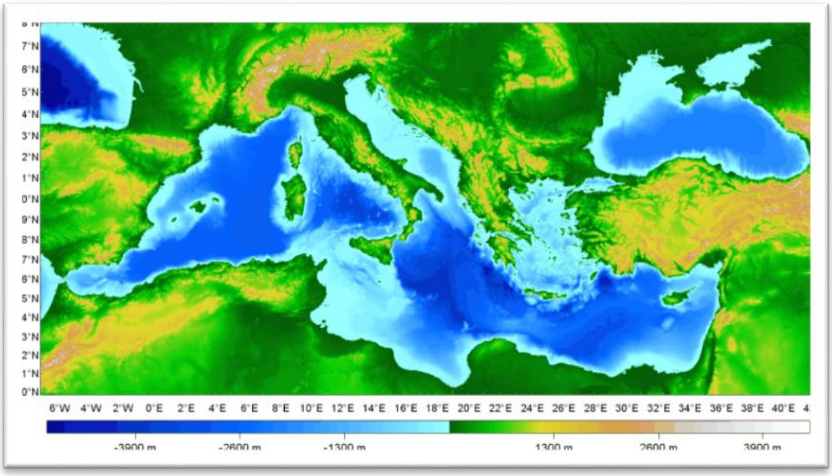


Figura 1 Mapa topográfico y batimétrico de la Cuenca Mediterranea (OceanMap, www-3.unipv.it).

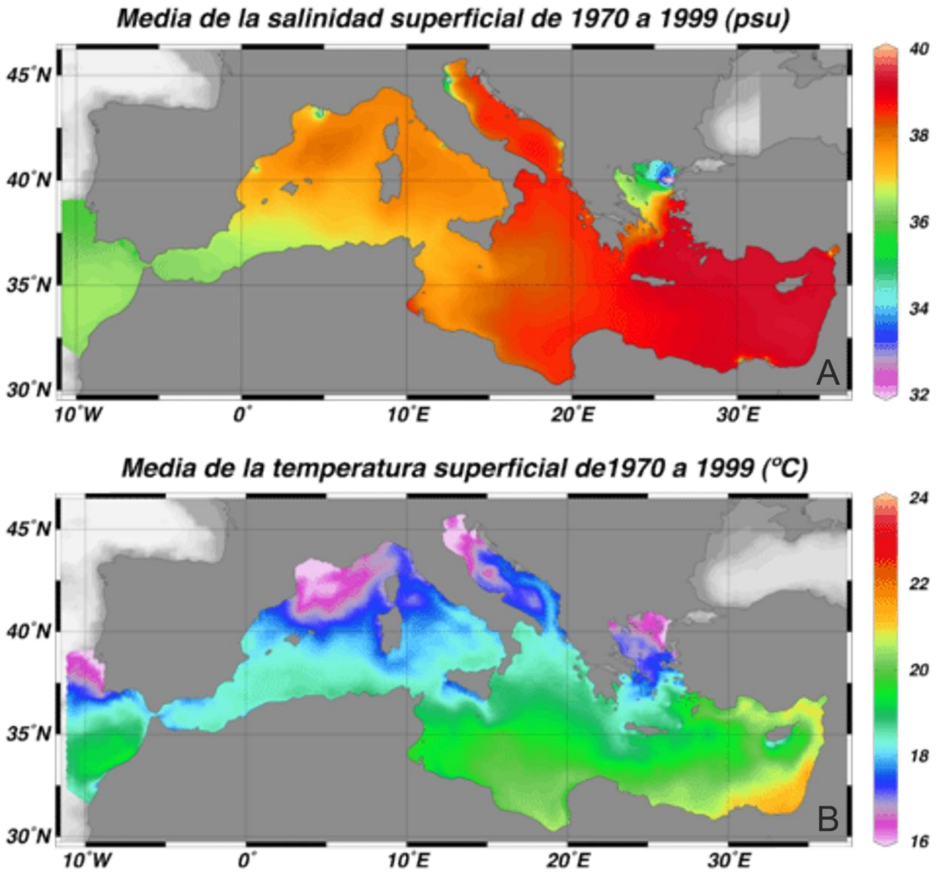
Muchas de las características hidrográficas del mar Mediterráneo son consecuencia de esta situación de mar casi interior y de las limitaciones en los intercambios entre subcuencas. En cierta manera, el Mediterráneo es considerado como un pequeño océano o, debido a la insignificancia de las mareas, un extenso lago de costas irregulares y abierto por ambos extremos. Los intercambios de agua con ambos océanos, Atlántico e Índico, son desiguales. El intercambio con el Atlántico es muy superior y de mayor efecto que el que ocurre a través del canal de Suez, si bien, los efectos ecológicos de la apertura del canal sobre la cuenca occidental han sido en algunos casos importantes (Lazzari et al., 2012; Rodrıguez, 1982).

Climatológicamente, el mar Mediterráneo se encuentra localizado entre zonas templadas y subtropicales, con temperaturas medias que oscilan entre aproximadamente 12°°°C de mínima y una máxima 25°°C en verano. Al estar rodeado por masas continentales de amplia extensión, presenta una climatología propia caracterizada por veranos cálidos y secos, inviernos moderados y un periodo que concentra buena parte de las lluvias durante la primavera. El factor más característico de este clima es su marcada estacionalidad (Lazzari et al., 2016; Rodrıguez, 1982). Durante el invierno la región se ve habitualmente afectada por los vientos del Oeste propios de las latitudes medias y es recorrida por numerosas perturbaciones portadoras de inestabilidad, vientos y lluvia. En cambio, durante el verano, las regiones mediterráneas quedan dentro del radio de acción de los anticiclones tropicales y subtropicales por lo que el aire es cálido y seco y las precipitaciones escasas. Esta dualidad en las características climáticas tiene repercusión sobre el funcionamiento de los ecosistemas marinos que deben afrontar condiciones muy variables durante el invierno y largas épocas de estabilidad en verano.

El mar Mediterráneo presenta un balance hidrológico negativo dado que se encuentra en un área donde la evaporación (∼1000 mm año^{-1}) excede a la precipitación (∼700 mm año^{-1}). Los aportes de los ríos son escasos (∼100 mm año^{-1}), resultando en una pérdida de agua media anual que oscila entre 500 a 700 mm y año^{-1} (Boukthir and Barnier, 2000; Mariotti et al., 2002). Este exceso de evaporación tiene como consecuencia una elevada salinidad (>36), por lo que es considerado el mar más salino de Europa (Fig. 2A). El déficit hídrico producido por el exceso de evaporación es compensado con aportes de agua desde el Atlántico oriental (0.72–0.92 Sv en promedio), lo que provoca un complejo sistema de corrientes e intercambio de agua en el estrecho de Gibraltar y Mar de Alborán (Borghini et al., 2014; Cardin et al., 2014; Malanotte-Rizzoli et al., 2013; Rodrıguez, 1982).

La temperatura de las capas superficiales varía entre los 13°°C de invierno y los 26°°C de verano, mientras que el agua profunda mantiene relativamente constante, unos

13°°C de media, siendo la cuenca oriental la más cálida (Fig. 2B). Como consecuencia de las variaciones estacionales en la temperatura superficial, durante la época estival, se produce una fuerte estratificación de la columna de agua que limita los intercambios verticales y, en consecuencia, la productividad de las aguas. Esta situación sólo se relaja a mediados de otoño cuando las primeras tormentas debilitan el gradiente térmico.



Media de la salinidad superficial de 1970 a 1999 (psu)

Media de la temperatura superficial de1970 a 1999 (°C)

Fuente: Tomado de Mediterráneo: más cálido y más salino, Roland Aznar (2011)

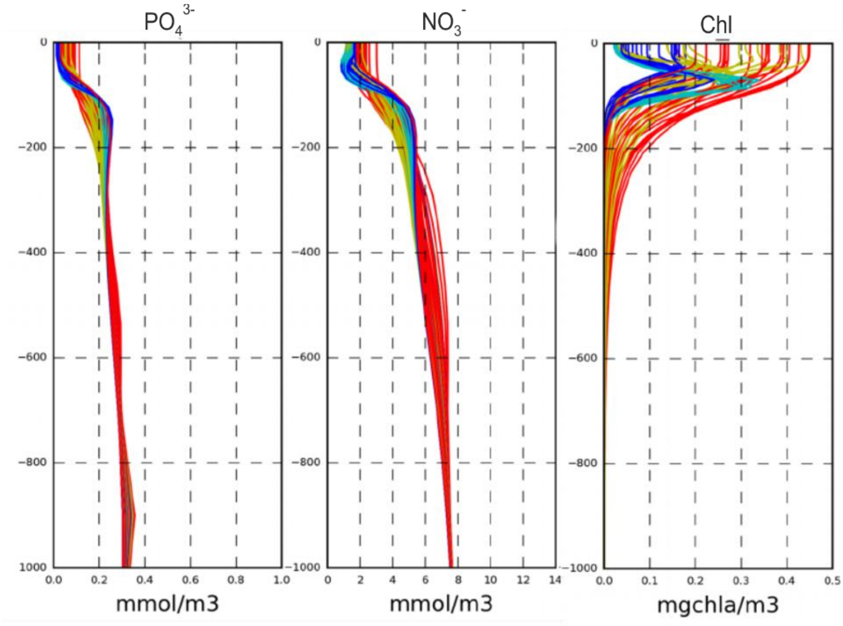
Figura 2 (A) Salinidad y (B) temperatura media superficial en el mar Mediterráneo.

Características biogeoquímicas

Producto de un balance hídrico negativo y la circulación del agua resultante, las aguas profundas mediterráneas exportan grandes cantidades de nutrientes al océano Atlántico que se pierden para la producción primaria interna (Goffredo and Dubinsky, 2013; Hopkins, 1985). El limitado suministro de nutrientes a las aguas superficiales del mar Mediterráneo, tanto desde sus capas más profundas como de fuentes externas, no compensa la exportación de nutrientes y otros elementos a las zonas más profundas. Este balance negativo se refleja en la distribución de nutrientes, metales y otros elementos (Fig. 3). La principal fuente de nutrientes en la zona eufótica es la mezcla invernal, si bien, en ciertas zonas puede haber otras formas de fertilización adicionales asociadas fundamentalmente a procesos de mesoescala que crean gran heterogeneidad espacial y temporal en este mar (Estrada, 1996; Estrada and Margalef, 1988; Estrada and Salat, 1989; Pinot et al., 1995).

A parte de los procesos de intercambio y mezcla, las principales fuentes de nutrientes y metales traza disueltos en el Mediterráneo son, (1) los aportes fluviales, (2) la descarga submarina de aguas subterráneas y 3) la deposición de aerosoles atmosféricos (Guerzoni et al., 1999; Krom et al., 2004; Ludwig et al., 2009; Rodellas et al., 2015; Tovar-Sánchez et al., 2014). Los ríos y otras fuentes de agua continental como las aguas subterráneas inyectan unos ~330 km^3 año^{-1} de agua dulce en el mar Mediterráneo (Boukthir and Barnier, 2000; Buddemeier, 1996), aproximadamente la mitad de este valor (~170 km^3 año^{-1}) es introducido por los 10 ríos más grandes que son una fuente importante de nutrientes (Ludwig et al., 2010). Así, las zonas más productivas de este mar se encuentran en las desembocaduras de los grandes ríos como el Ródano, el Po, el Danubio o el Ebro (Béthoux et al., 1998; Lefevre et al., 1997; Ludwig et al., 2009; Pujo-Pay et al., 2006; Pujo-Pay and Conan, 2003). Según Ludwig et al. (2010) en el año 2000 estos ríos aportaban una media de 923 kt yr^{-1} de N-NO$_3^-$ y 62 kt yr^{-1} de P-PO$_4^{3-}$, enriqueciendo las aguas de los estuarios, deltas y plataformas adyacentes. La creciente necesidad de agua para el consumo en los países Mediterráneos, y el desarrollo de la agricultura y la industria ha dado lugar al represamiento de los cauces más importantes, y la consecuente reducción de los aportes de agua y nutrientes de los ríos (Tovar-Sánchez et al., 2016).

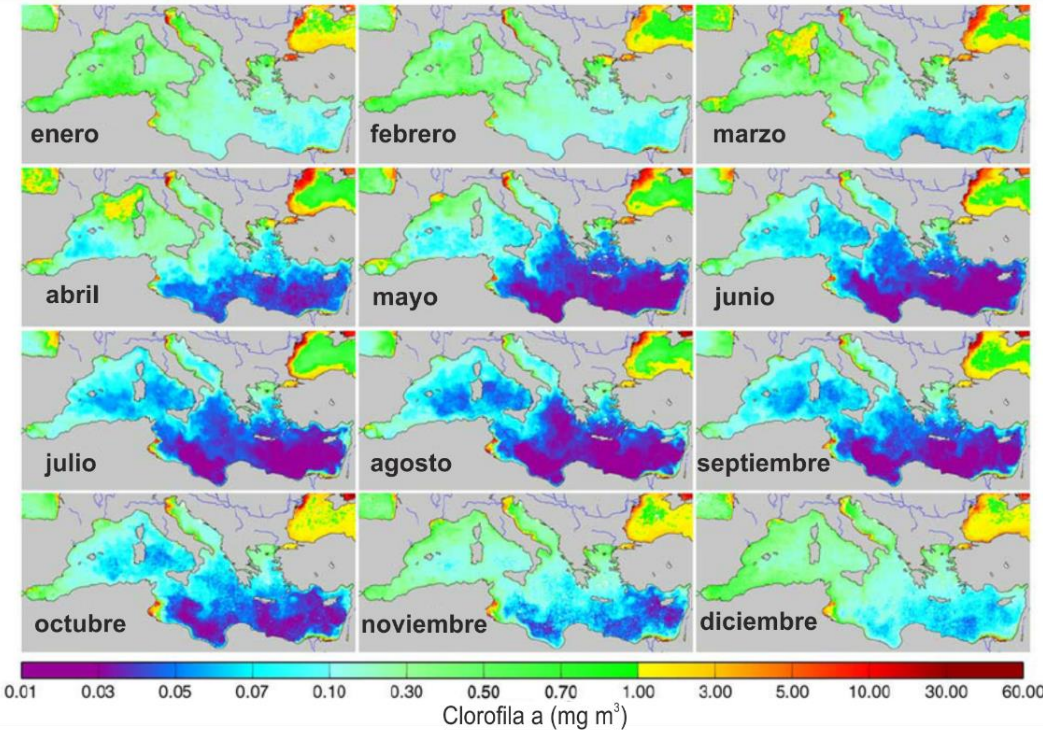
En las zonas alejadas de las desembocaduras de los ríos, los aportes de agua subterránea son una fuente importante de nutrientes y otros elementos. La descarga submarina de aguas subterráneas de los acuíferos costeros, que se estima en 2.200 m^3 s^{-1}, representa casi una quinta parte de la afluencia total de agua dulce en el Mediterráneo, con más de un tercio de esta descarga que entra desde las costas europeas del mar (Zektser et al., 2006). Muchos de estos acuíferos costeros reciben efluentes agrícolas así como residuos industriales y urbanos. Las descargas submarinas de agua subterránea (*Submarine Groundwater Discharges,* SGD) es una fuente relevante de nutrientes y metales traza disueltos en el mar Mediterráneo llegando a ser en algunos casos comparables a los aportes de ríos y deposiciones atmosféricas (Beck et al., 2007; Garcia-Solsona et al., 2009; Rodellas et al., 2015; Tovar-Sánchez et al., 2014; Windom et al., 2006).



Fuente: Tomado de Lazzari et al. (2016)

Figura 3 Perfiles verticales (hasta 1000 m) de fosfatos, nitratos y clorofila total correspondientes al Mediterráneo noroccidental. Las líneas rojas, amarillas, cian y azul se refieren, a los perfiles de invierno, primavera, verano y otoño respectivamente.

Las concentraciones de las formas inorgánicas de nutrientes en aguas del Mediterráneo oscilan entre 0,05 a 0,20 µM para P-PO_4^{3-}, de 1 a 4 µM para el N-NO_3^- y aproximadamente 1,2 µM para silicato (Coste et al., 1988; Lazzari et al., 2016). Además, existe un claro gradiente oeste-este en las concentraciones de nutrientes superficiales (Lazzari et al., 2016, 2012). Las bajas concentraciones de nutrientes superficiales tienen como consecuencia una limitación de la producción primaria que afecta de forma más intensa al Mediterráneo oriental. Sin embargo, también hay una significativa limitación de nutrientes en su parte occidental. Cuando se produce la limitación de nutrientes, en la gran mayoría de los casos, el fósforo es el limitante, con la notable excepción del Mar de Alborán, que está principalmente limitada por nitrógeno, y la cuenca del suroeste, en el que tanto el nitrógeno y el fósforo pueden limitar el crecimiento del plancton (Krom et al., 2004; Lazzari et al., 2016).



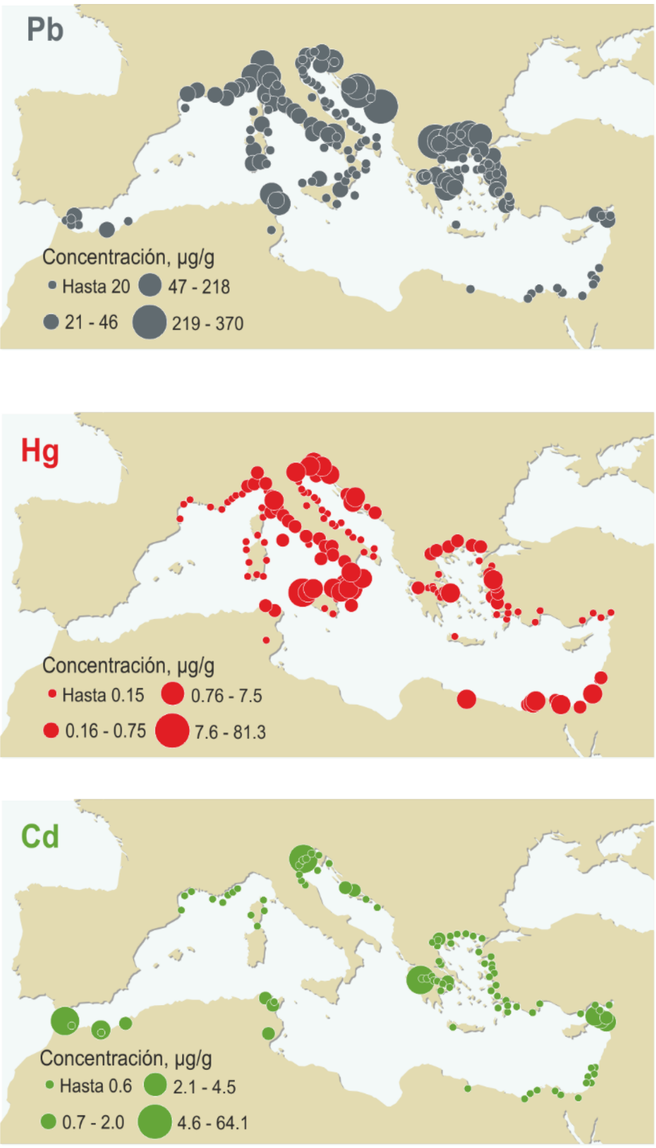
Fuente: Bosc et al. (2004) derivado de SeaWiFS data

Figura 4 Variación mensual de la concentración de Clorofila a en el mar Mediterráneo.

La escasez de nutrientes disueltos en superficie limita la producción primaria y la biomasa de fitoplancton, lo que hace que el mar Mediterráneo se considere, en general, un mar oligotrófico. Las concentraciones oceánicas de clorofila superficial varían entre 0.25 a 0.40 mg m-3 en invierno, pudiendo a menudo exceder los 1.00 mg m-3 en la región noroeste, y alrededor de 0.07 mg m-3 en verano (Fig. 4) (Bosc et al., 2004; Lavigne et al., 2015; Lazzari et al., 2012). En invierno o comienzos de la primavera, son características las floraciones fitoplanctónicas (‘blooms’) preoducidos por la mayor disponibilidad de nutrientes en la capa fótica (Bosc et al., 2004; Estrada, 1996; Mura et al., 1996; Ribera d'Alcalà et al., 2004; Siokou-Frangou et al., 2010; Zingone and Sarno, 2001). Estas suelen ocurrir primero en el sur y se desplazan progresivamente hacia el norte (Bosc et al., 2004). Una mayor irradiancia (20 % más) y una temperatura del agua más elevada (de 3 a 5 °C) en el Mediterráneo con respecto a latitudes parecidas en el Atlántico favorecen el incremento del fitoplancton en esta época (Duarte et al., 1999) pero debido a lo variable de las condiciones en esta época del año, estas proliferaciones suelen ser breves (Marty et al., 2002; Siokou-Frangou et al., 2010). Durante el otoño, cuando la columna de agua se enfría, también pueden ocurrir pequeños picos de biomasa fitoplanctónica. Sin embargo, la aparición de estas floraciones otoñales es muy desigual tanto a escala espacial como temporal (Bosc et al., 2004).

Por otro lado, la distribución de metales traza como Cd, Co, Cu, Mn, Ni y Pb disueltos en el mar Mediterráneo, al igual que en los océanos del mundo, están dominadas por la advección lateral y la mezcla vertical y muestran una disminución más o menos continua con la profundidad (Copin-Montegut et al., 1986; Morley et al., 1997; Riso et al., 2004, 1994; The Mermex Group, 2011). Las numerosas fuentes de emisión terrestres a lo largo de sus costas densamente pobladas (The Mermex Group, 2011), los intercambios a través de los estrechos de Gibraltar y Sicilia (Elbaz-Poulichet et al., 2001a, 2001b; The Mermex Group, 2011) y la deposición atmosférica (Béthoux et al., 1990; Heimbürger et al., 2011; Migon et al., 2002; The Mermex Group, 2011), son los factores que controlan la distribución y ciclos biogeoquímicos de los metales traza en el mar Mediterráneo. Elbaz-Poulichet et al., (2001b) sugieren que, la principal fuente de metales disueltos (As, Cd, Co, Cu, Fe, Mn, Ni, Pb y Zn) en el Mediterráneo occidental la constituyen las entradas a través de los estrechos, en segundo lugar, la deposición atmosférica y que los ríos constituyen la menor de las fuentes. Los aportes atmosféricos en el mar Mediterráneo, sobre todo al noroeste, se originan a partir de fuentes de emisiones naturales y antropogénicas, en las que los eventos episódicos, pero intensos, de polvo del Sahara constituyen la fuente principal de elementos traza (Chester et al., 1997; Guerzoni et al., 1999; Guieu et al., 2002; Heimbürger et al., 2011; Marty et al., 2002; Ternon et al., 2010). El clima y la meteorología del mar Mediterráneo determinan la forma (disuelto o particulado) en que estos metales entran a la superficie del mar (Nicolas et al., 1995). En el Mediterráneo las entradas de metales antropogénicos se realizan principalmente por medio de las masas de aire del norte y centro de Europa. Según Morley et al., (1997) como resultado de estas entradas atmosféricas, las concentraciones de metales en las aguas superficiales del Mediterráneo son más altas que en el océano abierto.

A lo largo de toda la cuenca mediterránea occidental, Martin et al., (1989) estimaron que las aportaciones atmosféricas de Pb, Cd y Cu eran dos a tres veces mayores que las de los ríos. Por su parte Guieu et al., (1997) y Guerzoni et al., (1999) obtuvieron factores similares para el Cd, Zn y Pb para la cuenca del noroeste. Elbaz-Poulichet et al., (2001a) estimaron flujos atmosféricos de Cu, Cd y Pb más bajos que los flujos previamente publicados por otros autores (Guerzoni et al., 1999; Guieu et al., 1997; Martin et al., 1989). No obstante, sus resultados confirman el predominio del flujo atmosférica sobre el flujo de ríos publicado por Guieu et al., (1997). En el caso particular del Hg, diversos autores han estimado que el aporte por deposición atmosférica puede ser hasta de un orden de magnitud mayor que el estimado para las entradas anuales de los ríos de todo el Mediterráneo (Cossa et al., 1997; Horvat et al., 2003; Rajar et al., 2007). Sin embargo, hay que tener en cuenta que, aunque las descargas fluviales son consideradas las fuentes menos importantes de metales, existen estudios que demuestran que en la zona costera tienen gran influencia, constituyendo la principal fuente de metales (Alonso Castillo et al., 2013; Dassenakis et al., 1997; Giani et al., 1994; Guieu et al., 1998; Oursel et al., 2014a, 2014b; Puig et al., 1999). También, Nicolau et al., (2012), en el río Eygoutier del Mediterráneo, encontró flujos medios hacia mar de Cu y Pb del mismo orden de magnitud que los flujos de otros ríos nord-mediterráneos y señalo que los pequeños ríos costeros pueden trasportar anualmente cantidades de estos metales del mismo orden de magnitud que el aportado anualmente por un gran río como el Ródano o Po.



Fuente: Tomado de State of the Mediterranean Marine and Coastal Environment (2013)

Figura 5 Concentración media de metales (Pb, Hg y Cd) en sedimentos de zonas costeras del mar Mediterráneo.

Zonas costeras mediterráneas

El mar Mediterráneo, en sus 46.000 Km de costa, alterna ecosistemas costeros de gran importancia ecológica como praderas de fanerógamas sumergidas, zonas rocosas intermareales y estuarios, entre otros. Estas zonas costeras tienen una gran diversidad de ambientes y recursos que las convierten en áreas especialmente atractivas para los asentamientos humanos, tanto como lugar de residencia como por la gran variedad de actividades productivas que se pueden implantar en ellas. Por ejemplo, se estima que más del 39% de la población española (15,6 millones de personas) viven en la costa del Mediterráneo (AEMA/PAM/PNUMA, 2007). Esto hace que las zonas costeras sean especialmente vulnerables, debido a que reciben impactos antropogénicos de actividades realizadas tanto en tierra como en el mar. Los principales factores antropogénicos que afectan las aguas costeras son el uso y explotación de recursos marinos, el desarrollo de infraestructuras, los asentamientos humanos y la urbanización, la industrialización, el turismo, el transporte marítimo, el dragado de puertos y el vertido de resíduos, entre otros (EEA, 2005; Halpern et al., 2008). Estas actividades provocan un aumento notable en los aportes de nutrientes y metales al medio acuático.

Las concentraciones de nitrógeno (N), fósforo (P) y silicio (Si) en la columna de agua están fuertemente correlacionadas con la producción primaria y las concentraciones medias de clorofila en los ecosistemas marinos costeros (Conley and Malone, 1992; Howarth, 1988; Ryther and Dunstan, 1971; Smith, 2006). El nutriente que limita el crecimiento del fitoplancton varía dependiendo de la zona de estudio e incluso de las condiciones ambientales que en ella se den. Generalmente, el nitrógeno es el nutriente limitante para el crecimiento y la producción de las poblaciones de fitoplancton de los ecosistemas costeros (Zohary and Robarts, 1998). Sin embargo, en el caso de las zonas costeras mediterráneas, existen evidencias que demuestran una alternancia espacio temporal entre nitrógeno y fosforo, llegando a ser en algunos casos el fósforo el nutriente limitante principal. Por ejemplo, en el este y noroeste del Mediterráneo Vollenweider et al., (1996), Krom et al., (1991), Estrada, (1996) y Thingstad et al., (1998), encontraron limitación por fosforo. Otros estudios sugieren que el nitrógeno puede ser el principal elemento limitante en zonas costeras del Mediterráneo occidental (Ribera d'Alcalà et al., 2004; Thyssen et al., 2008). Marty et al., (2002) detectaron un cambio de limitación por fósforo en la época oligotrófica a limitación por nitrógeno en invierno en el Mar de Liguria. Por el contrario, Charles et al., (2005) observaron en la costa francesa mediterránea una limitación por nitrógeno en verano, mientras que el resto de épocas estaba limitado principalmente por fósforo. En la Bahía de Andratx (Isla de Mallorca), Puigserver, (2003) encontró una limitación alternante por nitrógeno o fósforo y una limitación por fosfato en el resto del ciclo estacional. Más recientemente, Tanaka et al., (2011) en estudios con microcosmos realizados en la época estival, observaron que la producción primaria estaba firmemente limitada por nitrógeno y, en menor medida, por fósforo.

Las zonas costeras de todo el mundo están sometidas a un cada vez mayor aporte de nutrientes relacionado con las actividades humanas. Estos aportes incluyen vertidos directos y también aquellos que alcanzan las aguas costeras mediante los ríos, el agua subterránea y/o la deposición atmosférica. Estas entradas no sólo proceden del continente, puesto que algunas actividades antrópicas se sitúan directamente en el medio marino. Existen evidencias que indican que la carga de P en las aguas marinas se ha incrementado multiplicándose por 3 en comparación con los niveles preindustriales y a la implantación de la agricultura intensiva; y se estima que el flujo de N se ha incrementado aún más (Howarth et al., 2000). En la bahía de Brest (Francia), se ha encontrado que la carga de nitrato se ha doblado respecto a la de los años 70, algo que está relacionado con el incremento de actividades agrícolas (Le Pape et al., 1996). Del mismo modo, Béthoux et al., (1992), encontró un incremento de las concentraciones de fosfato y nitrato en las aguas profundas de la cuenca oeste del mar Mediterráneo debido a un incremento en la agricultura y en las actividades industriales y urbanas durante los años 60. Estos registros de nutrientes proporcionan evidencias de un cambio rápido en la fertilidad de los ecosistemas costeros durante la última mitad del siglo XX (Cloern, 2001).

La actividad humana causa un importante impacto sobre los ecosistemas marinos costeros, provocando perturbaciones en el medio ambiente tanto de forma directa como indirecta. Las principales actividades que afectan al medio marino costero incluyen entre otros, la pesca, la acuicultura, el desarrollo urbanístico, el turismo, el transporte marítimo, la industria, la agricultura, (Barnabe and Barnabe-Quet, 2000; Halpern et al., 2008, 2007; Nogales et al., 2011; Shahidul Islam and Tanaka, 2004). En zonas cuya actividad económica está basada en el turismo, se añade la presión de una gran afluencia de turistas, sobre todo en los meses de verano. El aporte de nutrientes que se deriva de estas actividades puede superar la capacidad del ecosistema para su asimilación y es entonces cuando ocurre la degradación de la calidad del agua (eutrofización). Este sobre-enriquecimiento acarrea diversos impactos incluyendo un incremento de la turbidez con la consiguiente pérdida de vegetación acuática sumergida, deficiencia de oxígeno, disrupción del funcionamiento del ecosistema, pérdida de hábitat, pérdida de biodiversidad, cambios en cadenas alimenticias, y pérdidas en las capturas pesqueras y las proliferaciones de algas nocivas o HABs (*Harmful Algal Bloom*) (Caroppo et al., 2015; Cloern, 2001; Glibert et al., 2010; Rablais and Nixon, 2002). La eutrofización constituye un problema preocupante en ciertas áreas del mar Mediterráneo ("puntos calientes" / bahías semicerradas).

Los nutrientes y metales que son introducidos en el ecosistema costero afectan tanto a la columna de agua como a los sedimentos. La composición del sedimento depende de las condiciones hidrodinámicas y de los materiales aportados por los ríos y las diferentes descargas procedentes de la cuenca hidrográfica, y también por el mar (Kaiser et al., 2011). Estos pueden actuar como reservorios de diferentes las sustancias introducidas al medio (Crain et al., 2009), de modo que estas sustancias pueden volver a la columna de agua si hay una perturbación de los sedimentos. El efecto de estas perturbaciones sobre el medio marino es complejo, porque normalmente se produce la introducción simultánea de varios compuestos diferentes y, además, porque estos pueden tener diferentes efectos en el medio. El impacto de la perturbación depende de la magnitud y la escala de esta (desde el punto de vista temporal) y, también, de las características ambientales del lugar en el que ocurre. Las mareas, corrientes, propiedades ópticas del agua, la salinidad, la profundidad de la columna de agua y las condiciones meteorológicas y climáticas influyen sobre las consecuencias que produce determinada perturbación sobre el ecosistema (Bouma et al., 2005, 2005; Cloern, 2001; Cloern and Jassby, 2008; Paerl et al., 2006; Ysebaert et al., 2003, 2002).

El Programa de las Naciones Unidas para el Medio Ambiente estima que 650 millones de toneladas de aguas residuales (70% sin tratar), 60.000 toneladas de mercurio, 3.800 toneladas de plomo y 36.000 toneladas de fosfatos se vierten en el Mediterráneo cada año. De acuerdo con el inventario del National Baseline Budget (NBB), las principales emisiones atmosféricas de metales en los países mediterráneos están relacionadas con la industria del cemento (Hg, Cu), la producción de energía (As, Cd, Ni) y la industria de los metales (Pb, Zn). La deposición atmosférica es la principal vía de entrada de metales pesados en aguas abiertas. Es dudoso que el impacto negativo de los aerosoles de origen antropogénico se limite al fitoplancton marino. Más probablemente, los efectos nocivos abarcarán a varios procesos del ecosistema marino y se extenderán a través de los ciclos biogeoquímicos (Jordi et al., 2012).

La contaminación también llega a las costas Mediterráneas a través de sus principales sistemas fluviales: el Po, Ebro, Nilo, y Ródano que transportan grandes cantidades de desechos agrícolas e industriales que aportan grandes cantidades de nutrientes y metales traza. Las principales descargas de metales en estas aguas parecen estar relacionados con la industria de los fertilizantes (Hg, As, Pb), industria del metal (Ni, Zn) y plantas de tratamiento de aguas residuales (Cd, Cu), con contribuciones importantes también del sector energético y la industria química (UNEP/MAP, 2010).

La elevada presencia de metales en la geología local también puede influir en el contenido de metales en las zonas costeras. Con independencia del tipo de fuente de metales de origen terrestre, los sedimentos costeros contaminados constituyen una fuente importante de contaminación secundaria difusa, al emitir metales hacia el agua que los cubre (Fig. 5) (AEMA/PAM/PNUMA, 2007). En el Golfo de Trieste se ha observado la emisión de metales (Cd y Cu) desde el sedimento contaminado hacia el agua que lo cubre (Zago et al., 2000).

Zonas costeras con intercambio restringido en el Mediterráneo

La vulnerabilidad de las zonas costeras ante los potenciales impactos naturales y/o antrópicos depende de sus características geológicas, morfológicas, ecológicas y ambientales. En general, los ecosistemas costeros siempre estarán impactados tanto natural como antropogénicamente. Ese impacto podrá ser permanente o el ecosistema podrá retornar a su estado inicial (no necesariamente por el mismo camino), dependiendo su capacidad de renovación y expulsión de las sustancias contaminantes (Perillo and Piccolo, 2011). Las zonas con mayor potencial de impacto, tanto por su naturaleza como por los asentamientos que soportan, suelen ser los estuarios, bahías y rías. Los estuarios se ven continuamente amenazados por vertidos de petróleo, dragados y rellenos de tierra dirigidos a la expansión industrial y residencial. Por su parte, la construcción de puertos y la contaminación que estos generan también han contribuido a deteriorar el estado de muchos ecosistemas.

Las bahías, estuarios, y otras aguas protegidas son los sitios por excelencia para el desarrollo urbano, turístico, industrial y la acuicultura. Estas zonas poco profundas, presentan un aumento en la productividad en relación con las aguas costeras adyacentes porque actúan como 'trampas' de nutrientes y/o partículas orgánicas que provienen tanto de tierra por medio de torrentes o transportados por el mar que amenazan la salud del ecosistema (Tett et al., 2003). Las áreas costeras, que reciben por partida doble cargas de nutrientes de origen antropogénico procedentes de los ríos y de los vertidos directos al mar de aguas residuales industriales y domésticas, son las más susceptibles de eutrofización. Podemos encontrar ejemplos de ello en lagunas costeras, estuarios y bahías semi-cerradas, como es el caso del Delta del Ebro, la Albufera de Valencia, lagunas costeras del sureste de Francia, la laguna de Túnez, la bahía Kastela en Croacia, la bahía de Izmir en Turquía, entre otros. (Bachelet et al., 2000; Dauvin et al., 2007; Estrada, 1993; Kharroubi et al., 2012; Kucuksezgin et al., 2006; Pusceddu et al., 2005; Vukadin and Stojanoski, 2004). Además, se observan elevados niveles de contaminantes como mercurio, cadmio, zinc y plomo en muestras de zonas costeras que reciben efluentes industriales, residuos sólidos y basuras domésticas (Er-Raioui, 2012; Oursel et al., 2014a, 2014b, 2013; Ruiz, 2001; Sánchez-Chardi et al., 2007; Sanchiz et al., 2000; Shahidul Islam and Tanaka, 2004; Zaqoot et al., 2012).

Los estuarios son ecosistemas acuáticos que forman zonas de transición entre el mar, el río, la tierra y la atmósfera, lo cual determina su singularidad en relación con la existencia de fuertes gradientes de las condiciones físicas y químicas y, por tanto, también de las comunidades biológicas asociadas (Attrill and Rundle, 2002; Edgar et al., 1999). Como consecuencia de ello, poseen tanto características de las aguas dulces como de las salinas, pero también presentan características diferenciales con las de los medios anteriores y propias de sí mismos. Algunas de estas propiedades únicas son comunes a todos los estuarios del mundo mientras que otras están determinadas por las condiciones locales de cada estuario. Una definición clásica utilizada para los estuarios, es la propuesta por Pritchard, (1967): "Un estuario es una masa de agua costera semi-cerrada que tiene una conexión libre con el mar y dentro del cual el agua marina está parcialmente diluida con el agua dulce que proviene del drenaje continental". Esta definición funciona bien para los estuarios de las zonas templadas donde estos están ligados a la boca de los ríos, pero no incluye cuerpos de salinidad altamente anómala como son lagunas, o entradas costeras que están conectados al océano solo ocasionalmente.

Existen diversos tipos de clasificaciones de los estuarios, generalmente basadas en los patrones de circulación del agua, las características de distribución de la salinidad y la geomorfología (Edgar et al., 1999). Una de las clasificaciones es basada en el balance entre el agua dulce y el agua salada, que determina la distribución vertical de la salinidad (Cameron and Pritchard, 1963). La amplitud de la marea también ha sido utilizada como factor discriminante para clasificar los estuarios en micromareales, mesomareales y macromareales (Hayes, 1975). Por otro lado, con base en la geomorfología, se distinguen cuatro tipos básicos de estuario: de llanura costera, de barrera, fiordos y tectónicos (Pritchard, 1960). Dichas clasificaciones han sido posteriormente modificadas y ampliadas por diferentes autores (Dalrymple et al., 1992; Davidson et al., 1991; Harris et al., 2002; Hume and Herdendorf, 1988; Perillo, 1995), desarrollando otras más complejas que combinan casi todas las características y/o factores que influyen en el origen y funcionamiento de los estuarios. Quizás la clasificación comúnmente más utilizada es la basada en sus propiedades de circulación y la distribución asociada de estado continuo de la salinidad. En este caso el tipo de estuario estará determinado por el cociente entre la entrada de agua dulce y el agua de mar mezclados por las mareas y se pueden clasificar en estuarios de cuña salina, altamente estratificado, ligeramente estratificado, mezclado verticalmente, inverso e intermitente. (Cameron and Pritchard, 1963; Dyer, 1997; Tomczak, 2000).

La constante necesidad de establecer una definición que se adapte a los diferentes ambientes estuarinos, ha dado origen a la modificación y ampliación de la definición de estuario. Ibáñez i Martí, (1993) propone una definición más amplia que incorpora los estuarios micromareales que presentan unas características particulares: "Un estuario es un sistema fluvio-marino sometido a la influencia de las mareas y caracterizado por una entrada y una mezcla de agua marina y continental que son variables en el espacio y en el tiempo". Este autor enfatiza en que en algunos estuarios la entrada de agua de mar y/o continental se interrumpe en la estación seca y que en los estuarios con cuña salina el agua marina desaparece durante los períodos de caudal elevado. Otra definición más actualizada es la de Potter et al., (2010): "Un estuario es un cuerpo de agua costera parcialmente cerrado, ya sea de forma permanente o periódicamente abierta al mar, que recibe de forma periódica porte de agua dulce, por lo tanto, mientras su salinidad puede ser menor que la del agua de mar natural y varía de manera temporal y longitudinal, pero pueden convertirse en hipersalinos en regiones donde la pérdida de agua por evaporación son altas y las entradas de agua dulce y las mareas son insignificantes"

Estas últimas definiciones dan lugar a un tipo de clasificación que se conoce como estuario intermitente, que se refiere a estuarios que pueden cambiar su tipo de clasificación debido a la alta variabilidad de la precipitación sobre el área de la cuenca de drenaje de su suministro fluvial. Aunque la entrada de agua dulce sea muy poca su carácter estuarino se mantiene. Pero, si el suministro fluvial se seca completamente durante la temporada de sequía, el estuario pierde su identidad y se convierte en bahía. Un ejemplo es el South West Arm de Puerto Hacking, al sur de Sydney, el cual se convierte en un estuario altamente estratificado en las semanas posteriores a fuertes precipitaciones. Este tipo de estuarios intermitentes se corresponde con estuarios de morfología semicerrada y flujos de intercambio restringido que se encuentran en ambientes mediterráneos como el que describe y define Largier, (2010).

A nivel mundial existen muchos estuarios que experimentan flujos de intercambio restringidos que exhiben una marcada variabilidad estacional (Cooper, 2001; Largier et al., 1997; Newton and Mudge, 2003; Sutula et al., 2003; Tett et al., 2003; Williams and Orr, 2002). Normalmente alterna entre un estado de estuario clásico (con flujo persistente de volúmenes significativos de agua dulce) en la estación húmeda y un estado inverso en la estación seca, que puede ser interrumpido por eventos episódicos de precipitación. En este tipo de sistemas el flujo restringido tiene como consecuencia la reducción de la circulación estuarina y el aumento de los tiempos de residencia. La profundidad de estos sistemas también es un parámetro importante, ya que en aguas pocos profundas las perturbaciones pueden ser significativas y los flujos longitudinales están reducidos. Largier et al, (1996, 1997, 2000) sugieren que en los estuarios de flujo restringido el intercambio longitudinal está dominado por la difusión de las mareas, por lo que la difusividad longitudinal y el tiempo de residencia se incrementan con la distancia desde interior hacia la boca del sistema. Sin embargo, incluso si la naturaleza del intercambio longitudinal es diferente, se puede observar aguas menos modificadas cerca de la desembocadura y las aguas más modificados en la cabecera de la cuenca.

Estudios sobre la diversidad de los estuarios en las últimas décadas, han hecho evidente que los estuarios con períodos de flujo restringidos son tan comunes como los estuarios clásicos. Largier (2010) sugiere que, en cualquier lugar en el que la evaporación exceda a la precipitación durante un tiempo más largo que el tiempo de residencia de la cuenca (por ejemplo, durante la estación seca), es posible encontrar estuarios de flujo restringido asociados a cuencas pequeñas y con poco volumen de almacenamiento (donde la escorrentía se aproxima a cero durante la estación seca) y que una breve revisión de los mapas estacionales de precipitación revela que estos estuarios se pueden encontrar a lo largo de muchas costas del mundo. El tipo de estuario de intercambio restringido de ambiente mediterráneo no solo se encuentran en España, Portugal, Marruecos y gran parte de las costas del mar Mediterráneo, sino que también se pueden encontrar en costas occidentales templados de latitud media caracterizados por tener climas mediterráneos o semiáridos, con veranos secos largos, como por ejemplo: California (USA), Baja California (Mexico), Suráfrica, el oeste de Australia y Chile (Largier, 2010).

Los estuarios mediterráneos presentan muchas características ecológicas comunes, en su mayoría relacionados con los tiempos de residencia largos observados en estas cuencas. Se sabe que la dinámica físico-química y las características ecológicas de los estuarios están fuertemente influenciadas por la descarga de agua dulce desde la tierra y el intercambio de agua con el océano adyacente (Gobler et al., 2005). Los estuarios son sitios claves en donde los procesos biogeoquímicos modifican los flujos de nutrientes y metales traza desde el medio terrestre hacia el mar (Falco et al., 2010; Jahnke et al., 2008). Se sabe que las aguas costeras, en comparación con mar abierto, están enriquecidas en nutrientes y metales trazas (Jonge et al., 2002; Kremling and Hydes, 1988; Kremling and Pohl, 1989; Le Gall et al., 1999; Nixon, 1995; Paerl, 1997; Pelley, 1998). Por lo tanto, hay una gran necesidad de entender los procesos que impulsan estos cambios en los ecosistemas costeros en una amplia gama de escalas temporales y espaciales.

Los estuarios presentan diferentes procesos físico-químicos en función de los aportes que reciben, la conectividad de sus aguas con el medio marino y su variación temporal. Estos procesos determinan la dinámica de los nutrientes y metales y los efectos de estos elementos sobre los organismos que lo habitan. La dinámica de nutrientes y metales en los estuarios mediterráneos no se conoce bien y se carece de información sobre las fuentes y procesos que controlan su movilización y destino. En el caso de los nutrientes, se asume que estos procesos que rigen su mezcla y destino en estuarios mediterráneos son diferentes a las que se producen en los estuarios clásicos (Delgadillo-Hinojosa et al., 2008). Algunos estudios han relacionado patrones longitudinales en los niveles de nutrientes (Camacho-Ibar et al., n.d.; Smith et al., 1991) y las concentraciones de fitoplancton (Kimbro et al., 2009) con los patrones longitudinales en los tiempos de residencia y de las tasas intercambio. Sin embargo, a pesar de la importancia de las perturbaciones naturales o antrópicas y los procesos fisicoquímicos que regulan la biogeoquímica del ecosistema, hay una falta de información sobre estos procesos que afectan a nutrientes y metales traza en estuarios de intercambio restringido.

Mahón. La zona de estudio

El Puerto de Mahón (Menorca) es considerado como uno de los mayores puertos naturales del Mar Mediterráneo. Presenta una longitud de aproximadamente 5,5 km, una anchura de ~0,6 km y una profundidad de hasta 30 m. La geomorfología de la zona está condicionada por una falla geológica con dirección NW-SE inundada por el mar. La cuenca interior es poco profunda, con una profundidad media de < 12 m, y alberga un puerto comercial en su orilla sur, al pie de la ciudad de Mahón (Moll de Ponent). La orilla norte está ocupada por una central térmica y unas instalaciones militares (Cós Nou y Estación Naval). La parte central del puerto es más profunda y ancha. La falla al sur de la Isla del Rey, con una profundidad superior a los 15 m. esta sección central discurre entre el Club Náutico y Es Castell, que es el segundo núcleo poblacional del estuario. La desembocadura del puerto es muy estrecha (~300 m) y relativamente poco profundas (~14 m), lo que limita en gran medida el intercambio con las aguas de la costa (Figura 6).

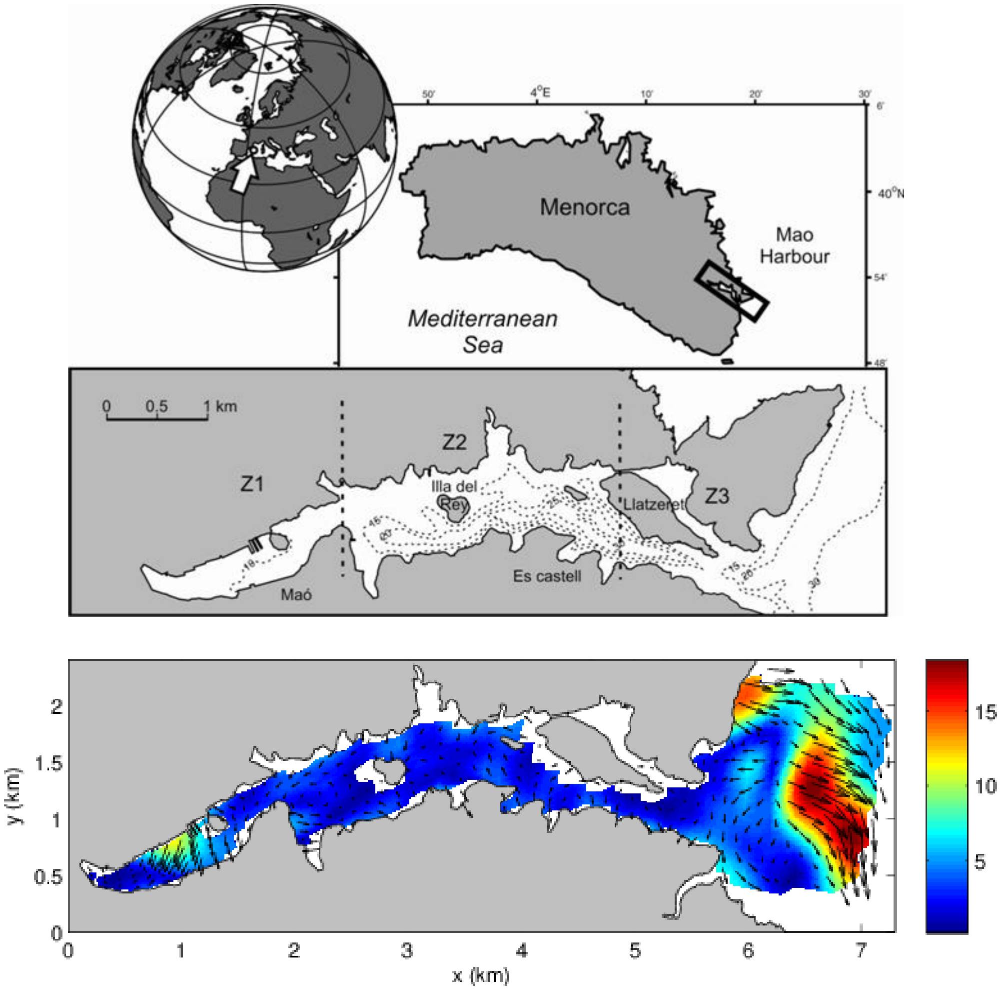


Figura 6 Mapa mundial que muestra el Mediterráneo Occidental, la isla de Menorca y el Puerto de Mahón. B) Distribución de la corriente superficial, revelando desacoplamiento entre la circulación en alta mar y los patrones de flujo internos del puerto. Intensidad de la corriente en cm s-1.

El clima de la zona se caracteriza por una fuerte estacionalidad, suaves inviernos húmedos (~13°°C) y con veranos calurosos y secos (~28°°C). La precipitación anual es de 563 ± 132 mm (datos de la Agencia Estatal de Meteorología, AEMET), si bien, existe una gran estacionalidad en régimen de lluvias. Así, la temporada de lluvias comienza entre fines del verano y principios del otoño. Los registros de la AEMET revelan una alta frecuencia de eventos de lluvia moderada (>10 mm) entre septiembre y diciembre, así como en primavera. De hecho, las precipitaciones durante el otoño suele ser un importante contribuyente a la precipitación anual (40±11% del total de las precipitaciones entre octubre y diciembre). Por el contrario, las precipitaciones son muy raras desde junio hasta finales de agosto.

Las características de las aguas del Puerto de Mahón no corresponden a una simple intrusión de las aguas marinas. El puerto recibe los aportes de agua continental de diversas fuentes. La cuenca hidrográfica de esta zona costera se extiende hasta el norte y se canaliza a través de un pequeño torrente que vierte en la parte interna del puerto tras regar una zona de huertos (Torrent Gorg). Las entradas de agua pueden ser torrenciales durante el otoño y a principios del invierno cuando la precipitación es mayor. Asimismo, se recibe aportes por escorrentía, pérdidas en las redes urbanas y descargas submarinas de aguas subterráneas. Éstas ocurren fundamentalmente a lo largo de la costa sur que presenta una litología de caliza.

El asentamiento humano en el entorno de Mahón es anterior a la época de los fenicios y, como es natural, la presión humana sobre sus aguas ha variado históricamente. Además de su utilización como enclave militar durante los siglos XVII-XVIII, también ha concentrado distintos tipos de industria, principalmente textil, joyería y calzado. Los efectos de esta actividad han dejado huella en los sedimentos del puerto, tal como se ha indicado en trabajos previos (Garcia-Orellana et al., 2011).

Hoy, el puerto de Mahón es un moderno puerto natural y técnicamente equipado, contando con un sector completo de reparaciones mecánicas y fábricas de la marina. Las excepcionales características geomorfológicas del puerto, su historia y su ubicación geográfica hace que sea un lugar con un alto atractivo turístico. Dos importantes zonas urbanas se asientan a lo largo de la costa sur, las poblaciones de Mahón y Es Castell con 30.000 y 8.000 habitantes respectivamente. Ambas presentan picos de población durante la temporada turística alta (Marí et al., 2004; OBSAM/IME, 2014)(Sergi et al., 2004; OBSAM y IME, 2014). Toda esta actividad tiene como consecuencia una presión en los sistemas de recogida de aguas residuales alrededor del puerto que, desde 1978, se vierten en su mayoría al mar abierto a través de un emisario.

Las actividades comerciales establecidas alrededor del puerto en la actualidad están representadas por las industrias de queso, calzado, joyas, metalurgia y electricidad térmica, turismo, deportes acuáticos, etc. Como resultado de esta actividad, barcos de crucero y mercantes de profundo calado transitan diariamente las aguas del puerto.

Todas estas características hacen que el puerto de Mahón sea un sitio importante desde el punto de vista biogeoquímico permitiendo estudiar cómo funcionan los procesos biogeoquímicos en un sistema de intercambio restringido, como se ven afectado estos procesos por factores naturales y/o antropogénicos y como su historia ha ido interviniendo en estos procesos.

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Objetivos

El puerto de Mahón tiene características interesantes desde el punto de vista biogeoquímico, por lo que se plantean las siguientes cuestiones:

- ¿Cómo se producen los intercambios entre el Puerto de Mahón y las aguas costeras? ¿Qué forzamientos las determinan?
- ¿En qué medida se ven afectados los procesos biogeoquímicos de Puerto de Mahón por factores naturales y/o antropogénicos?
- ¿Ha intervenido la historia comercial de este ecosistema en el funcionamiento de estos procesos?

La hipótesis es que el funcionamiento de los procesos biogeoquímicos de un ecosistema de intercambio restringido, como el puerto de Mahón, ha sido y esta está siendo inducido tanto por episodios naturales (tormentas costeras, lluvias intensas, variaciones en la descarga de aguas subterráneas u oscilaciones en el nivel del mar) y acciones antropogénicas resultantes de la propia actividad comercial del puerto.

Para comprobar dichas hipótesis se analizan los procesos y transformaciones biogeoquímicas que afectan al funcionamiento ecológico de un ecosistema costero mediterráneo con intercambio restringido con el mar (Puerto de Mahón). El estudio abarca los dos principales compartimentos ambientales: los sedimentos y la columna de agua. Para lograr este objetivo general se desarrollaron los siguientes objetivos específicos:

- Analizar la dinámica de las aguas del puerto, su estacionalidad y respuesta a forzamientos de episodios atmosféricos.
- Caracterizar la composición química de las aguas y sedimentos superficiales de Mahón y evaluar las posibles fuentes de nutrientes y metales traza.
- Evaluar la respuesta del fitoplancton marino a los distintos escenarios biogeoquímicos que ocurren estacionalmente en el ecosistema de Mahón.
- Identificar y describir los cambios históricos que ha sufrido el funcionamiento biogeoquímico de Mahón como consecuencia de las variaciones naturales y antropogénicas ocurridas en el último siglo.

Metodología General

Monitorización de las condiciones ambientales en el Puerto de Mahón. Datos hidrográficos y meteorológicos.

Las condiciones hidrográficas e hidrodinámicas en el Puerto de Mahón se monitorizaron mediante una serie de instrumentos oceanográficos que se mantuvieron instalados en distintas zonas del Puerto entre Junio de 2010 y Julio de 2011 (ver figura 7). Los datos meteorológicos se obtuvieron de la Agencia Estatal de Meteorología (AEMET) y corresponden al Aeropuerto de Mahón localizado a 3,5 km hacia el sur desde el área de estudio. También se obtuvieron datos adicionales de la estación meteorológica de la Autoridad del Puerto de Mahón, localizada en la boca del estuario. Ambas series temporales se fusionaron para obtener un registro continuo para el período de estudio.

La temperatura y salinidad superficial se midieron en intervalos de 1 minuto con dos SBE-CT instalados a aproximadamente 0,5 m bajo la superficie del agua. Las variaciones del nivel del mar se obtuvieron de la base de datos de Puertos del Estado. El mareógrafo, un radar de microondas MIROS, está situado en la isla Pinto y registra variaciones a intervalos de 5 minutos. Este instrumento es parte de la red de medidas del nivel del mar REDMAR.

Se obtuvieron perfiles de velocidad de corriente mediante perfiladores doppler ADCP (1000 kHz Nortek Aquadopp) en dos puntos del estuario (ver Figura 1). Las corrientes se midieron a intervalos verticales de 1 m, con el primer nivel de 1 m por encima del fondo. De acuerdo con la configuración de los otros equipos, el intervalo de muestreo temporal también se estableció a 1 minuto en ambos ADCPs.

Durante todo el periodo de muestreo se llevaron a cabo muestreos, en intervalos variables de 7 a 10 días, en los que se realizaban perfiles verticales de temperatura y salinidad en tres estaciones de muestreo (St1, St2 y St3; Fig. 1) utilizando un CTD YSI CastAway, además se obtenían muestras para el análisis de nutrientes, clorofila, fitoplancton y otras variables.

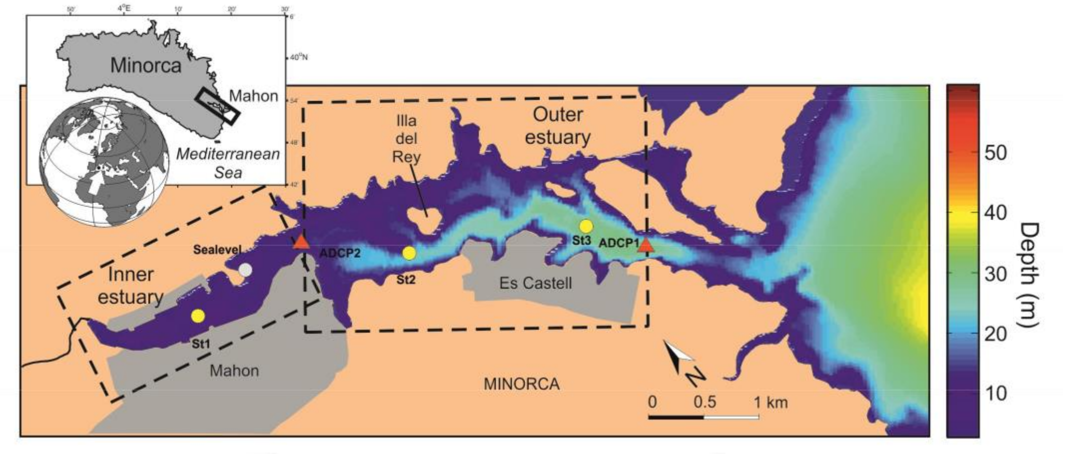

Figura 7 Mapa batimétrico (m) de Mahón. Los puntos amarillos representan las estaciones de las series temporales. Los triángulos rojos y el punto cian indican la posición de los ADCPs, el CT y el registrador del nivel del mar.

Para analizar las corrientes, estas se descompusieron en su componente a lo largo del estuario (u) y en la transversal a éste (v). Debido a que nuestro interés era la variabilidad submareal en el estuario y, en particular, la identificación de anomalías relacionadas con los distintos eventos meteorológicos que ocurrían en el estuario, se filtraron los datos mediante un filtro de 33h para eliminar las fluctuaciones con frecuencias diurnas o superiores.

El tiempo de renovación diaria (τf) para los dominios internos y externos del puerto de Mahón se estimaron a partir del intercambio de masa a través las secciones de control en las que se instalaron los correntímetros, asumiendo que el flujo era homogéneo en cada nivel de profundidad. Estos valores de τf representan una estimación del límite inferior de la renovación de las aguas del estuario. El valor real se supone algo más elevado ya que una parte del flujo se produce por movimientos oscilatorios.

Muestreos.

Los muestreos espaciales se realizaron durante cuatro campañas (Julio 2010, Octubre 2010, Marzo 2011 y Junio 2011). En cada una de ellas, se muestrearon 15 estaciones siguiendo el eje principal del puerto (Fig. 8). En cada estación se caracterizaron las propiedades hidrográficas de la columna de agua con CTD SBE-25. En cada punto se obtuvieron muestras de agua superficiales. Adicionalmente, se recogieron muestras de agua para el análisis de metales traza y nutrientes en el torrente que fluye hacia la parte interior del puerto (en Octubre de 2010 y Marzo de 2011) y en ocho pozos de aguas subterráneas cercanos a la costa del puerto (W1-8) (Fig. 8).

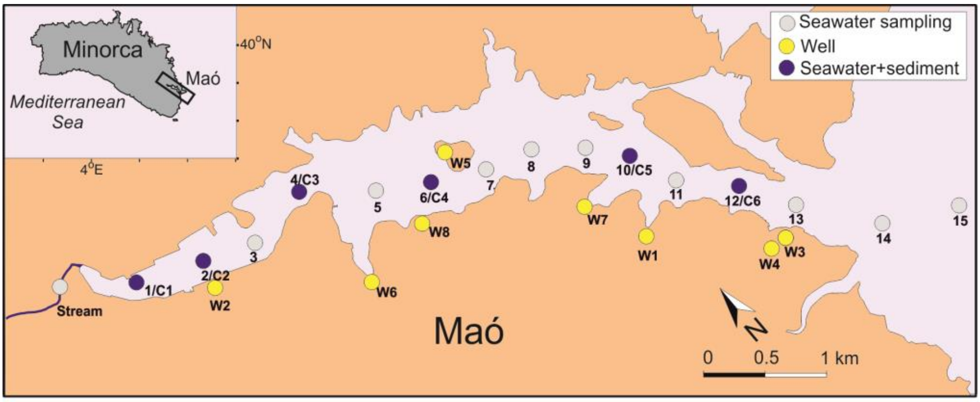

Figura 8 Mapa mostrando la ubicación del área de estudio en la isla de Menorca (Norte-Mediterráneo Occidental), y las estaciones de muestreo de aguas superficiales (1 a 15), pozos de aguas subterráneas (W1 a W8) y sedimentos (C1 a C6).

Las muestras de nutrientes fueron recolectadas por triplicado en tubos de polietileno (13 ml) lavados con ácido clorhídrico. En el caso de la clorofila, se filtró un máximo de 240 ml mediante filtros de fibra de vidrio GF/F (Whatman Ltd.) y después se congelaron hasta su análisis.

Para determinar los metales traza, se obtuvieron muestras de agua de 1 metro de profundidad utilizando una bomba peristáltica equipada con tubo C-Flex. El agua fue filtrada en línea a través de un filtro de cartucho de polipropileno (0.22 µm; MSI, Calyx®). Todo el material fue previamente lavado con ácido clorhídrico y siguiendo el protocolo de muestreo descrito por Tovar-Sánchez (2012).

Las muestras de sedimento superficiales (1-5cm) y testigos de sedimentos (hasta 55 cm de profundidad) fueron obtenido por buceadores en los puntos indicados en la figura 8. Las muestras de sedimento superficial se guardaron en tubos de ensayo de polietileno, mientras que las muestras de los testigos (estaciones C-3, C-4, C5 y C-6) se cortaron en secciones de 1 cm, y congeladas en tubos de ensayo de polietileno hasta su procesamiento en el laboratorio.

Análisis Químicos y Biológicos.

Nutrientes.

Las concentraciones de nitrógeno inorgánico disuelto (DIN: NO_3^-+ NO_2^-+NH_4^+), fosfato (PO_4^{3-}) y silicatos reactivos solubles (SiO_2) fueron medidas empleando un auto analizador marca Allianze modelo Futura, siguiendo los protocolos descritos por Grasshoff et al., (1983). El fósforo total disuelto (TP), fue estimado mediante la preoxidación de la muestra con un protocolo de digestión alcalina de persulfato que hidroliza todas las formas de fósforo inorgánico y orgánico a ortofosfato. El fósforo orgánico disuelto (DOP) fue estimado como la diferencia entre TP y PO_4^{3-}. Los análisis fueron certificados mediante el uso de material de referencia para nutrientes en agua de mar (MOOS-1, NRC-CNRC), obteniéndose recuperaciones del 97%, 93%, 98% y 118% para PO_4^{3-}, NO_3^-, NO_2^- y SiO_2, respectivamente. El límite de detección (LOD), calculado como tres veces la desviación estándar de la medida de los blancos, fue PO_4^{3-}: 0.04 µM, NO_3^-: 0.001 µM, NO_2^-: 0.001 µM, NH_4^+: 0.03 µM y SiO_2: 0.02 µM.

Clorofila.

Las concentraciones de Clorofila-a (Chla) se determinaron mediante un análisis fluorométrico de los pigmentos extraídos en acetona. Un volumen de muestra de entre 120 y 240 ml fue filtrado a través de filtros de fibra de vidrio (GF/F – Whatman) y almacenados a -20°°C (Parsons et al. 1984) hasta su procesado y análisis en el laboratorio. Los pigmentos se extrajeron en acetona al 90% y se almacenaron en la oscuridad a 4°°C durante toda la noche hasta su medición. La fluorescencia de los extractos se cuantificó empleando un fluorómetro Turner Designs calibrado con clorofila-a pura (Sigma Co.).

Metales traza.

Aguas.

Para el análisis de metales traza las muestras de agua fueron acidificadas a un pH < 2 con ácido clorhídrico (HCl) de la máxima pureza (ultrapure, Merck) en una campana de flujo laminar HEPA clase 100 y almacenadas por al menos un mes antes de su análisis. Los metales disueltos (<0.22 µm) (Cd, Co, Cu, Fe, Mo, Ni, Pb y Zn) fueron preconcentrados mediante el método de extracción orgánica APDC/DDDC (Bruland et al. 1979; Tovar-Sánchez, 2012) y analizados por espectrometría de masas con plasma de acoplamiento inductivo (ICP-MS) (PerkinElmer ELAN DRC-e). Los tratamientos y análisis fueron certificados mediante el uso de material de referencia para metales traza en aguas costeras (NASS-5, NRC-CNRC), obteniendo recuperaciones del 108%, 93%, 107%, 93%, 97%, 92%, 88% y 105% para Cd, Co, Cu, Fe, Mo, Ni, Pb y Zn, respectivamente. El límite de detección, calculado como tres veces la desviación estándar de la medida de los blancos fue 6, 4, 141, 129, 309, 22, 4, 427 y 287 pM para Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn, respectivamente.

Sedimentos.

Las muestras de sedimentos fueron secadas en una estufa a 60°°C por dos días y posteriormente pulverizadas en un molino de bolas de óxido de zirconio (10 minutos a 170 rpm). Las muestras fueron digeridas mediante digestión ácida en microondas (CEM modelo Mars 5), de acuerdo al SW-846 EPA método 3051A (US EPA 1987), el cual involucra la digestión, por triplicado, de 0.2 g de muestra de sedimento con 10 ml de ácido nítrico (HNO_3 65%, calidad Suprapur) en viales de teflón. Una vez digeridas las muestras fueron diluidas hasta 50 ml utilizando agua ultrapura (Mili-Q) para posteriormente ser analizadas por espectrometría de emisión con plasma acoplado inductivamente (ICP-EOS) (Perkin Elmer ICP-OES Optima 5300 DV). El método de extracción y análisis fue verificado mediante el uso de material certificado de referencia PACS-2 (National Research Council Canada), con recuperaciones de 99.4%, 99.9%, 90.9% 86.7%, 92.6 % 89.8%, 91.6% y 93.3% para Cd, Co, Cu, Fe, Mo, Ni, Pb y Zn respectivamente. Los límites de detección fueron calculados como tres veces la desviación estándar de los valores de los blancos siendo 0.6, 1.7, 0.8, 6.0, 4.0, 0.8, 17.5 y 2.0 $ng \cdot g^{-1}$ para Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn, respectivamente.

Granulometría.

El tamaño del grano en cada muestra de sedimento fue determinado mediante tamizado y posterior pesado de cada una de las fracciones obtenidas. Las muestras fueron tratadas previamente con peróxido de hidrógeno para remover la fracción orgánica (McManus, 1988; Walling y Woodward, 1993) y secadas en una estufa a 60°°C durante dos días. Una vez seca, se tamizó una muestra de aproximadamente 50 gr y se hizo vibrar durante 15 minutos para asegurar el paso del sedimento por todos los tamices. Las aberturas de malla utilizaras fueron: 2 mm, 1 mm, 500 µm, 250 µm, 125 µm y 63 µm. El material retenido en cada tamiz fue pesado para estimar el porcentaje de cada fracción.

Datación de sedimentos.

La determinación de actividad total de ^{210}Pb fue llevada a cabo mediante la medición de su nucleído emisor alfa descendiente ^{210}Po, siguiendo la metodología descrita por Sanchez-Cabeza et al. (1998). Posterior a la adición de ^{209}Po como un marcador interno, provisto por la Agencia Internacional de Energía Atómica (AIEA) (0.7031 ± 0.050 Bq ml1^{-1}), se digirieron muestras de alícuotas de 200-300 mg en su totalidad en un medio ácido, utilizando para ello una estufa de microondas analítica e isótopos de Po depositados en discos de plata en HCl 1 N a 70 °°C con agitación por 8 horas. Las emisiones de Po fueron posteriormente cuantificadas con espectrómetros-α equipados con detectores de silicio de barrera superficial con baja señal de fondo. Las recuperaciones químicas abarcaron entre 85 y 100% y las resoluciones de energía entre 20 y 35 keV. Las actividades de ^{137}Cs y ^{226}Ra fueron determinadas por espectrometría γ con un detector Ge coaxial de alta pureza (EG&G Ortec) calibrado con la solución estándar SRM-4276, provista por el National Institute of Standards and Technology. Las muestras fueron selladas por al menos 3 semanas previo al análisis, para permitir el equilibrio entre el ^{226}Ra y sus descendientes de vida corta. La actividad de ^{226}Ra fue determinada a partir del ^{214}Pb a través de su emisión de línea gama 351 keV. El exceso de ^{210}Pb (210Pbxs) fue estimado al sustraer la actividad de ^{226}Ra (que se asume representa la actividad soportada de ^{210}Pb) de la actividad total de ^{210}Pb. La calidad de las mediciones fue evaluada por la participación del laboratorio en pruebas de proficiencia IAEA y análisis continuos de materiales de referencia certificados y sus réplicas.

Actividad de la fosfatasa alcalina.

La actividad de la fosfatasa alcalina (APA) se midió mediante un ensayo cualitativo específico para células, utilizando enzimas marcadas con fluorescencia (ELF, por sus siglas en inglés) (Dyhrman and Palenik, 1999; Nicholson et al., 2006). Una vez marcadas, se identificaron las células mediante microscopía. De esta manera se pudo identificar qué comunidades de fitoplancton eran responsables de la actividad de fosfatasa alcalina.

Para determinar la APA en una base específica de la célula se requiere la adición de un sustrato de fosfomonoéster (ELF-97), que produce un precipitado fluorescente de color amarillo-verdoso donde se da la hidrólisis del éster, permitiendo el marcado de todas las células que expresen APA. Para ello, se filtraron 5 litros de agua de mar a través de un filtro Nytex de 100 µm para la eliminación de zooplancton, luego fue concentrada hasta 50 ml por filtrado inverso mediante un filtro Nytex de 20 µm. Una vez concentrada, la muestra fue filtrada por gravedad en una rampa utilizando un filtro Nytex de 20 µm de 25mm de diámetro, y posteriormente colocado en un tubo Eppendorf con 1.5 ml de etanol al 70%. La muestra fue incubada por al menos 30 minutos y posteriormente centrifugada a 2000 rpm (microcentrífuga) durante 15 minutos, para eliminar la mayor cantidad de etanol posible. Finalmente, se añadieron 100 ml de producto de ELF (kit de detección ELF97 de fosfatasa endógena) a la muestra, y se realizó una segunda incubación de 30 minutos antes de realizar el análisis de las células. El microfitoplancton total (de aquí en adelante el fitoplancton) y las células marcadas con ELF se identificaron y enumeraron utilizando una cámara de Sedgewick Rafter usando un microscopio de epifluorescencia Leica. Las células se identificaron usando iluminación de contraste de fase y luego se examinaron con una lámpara de mercurio de 100 W y un conjunto de filtros DAPI de paso largo (excitación de 350 nm, emisión de 520 nm) para determinar la APA en las células marcadas con ELF. Se estimó un coeficiente de variación promedio para los recuentos triplicados del 10% utilizando este método.Experimento sobre la respuesta de fitoplancton a la adición de PO_4^{3-}.

La escasez de fosfato en las aguas costeras puede ser limitante para el crecimiento fitoplanctónico. Se llevaron a cabo experimentos *in situ* para analizar la respuesta del fitoplancton a los aportes de fosfato que se pueden producir en Mahón y para estimar la importancia de la asimilación de DOP en los distintos grupos de microfitoplancton. El experimento consistió en la incubación de una serie de botellas de gran volumen de que contenían agua de mar y a las que se les añadió distintas concentraciones de fosfato. La incubación se prolongó durante 3 días y, cada 12 h se obtenía una muestra para ver la evolución de las comunidades contenidas en las botellas.

En primer lugar, se recogió agua de mar de la superficie de una estación de la zona externa de la bahía en bidones de plástico de 50 L previamente lavados con ácido clorhídrico, el agua fue pre-filtrada a través de una malla de 60 micras para excluir el zooplancton. Las incubaciones para cada tratamiento se realizaron por triplicado en botellas de polietileno de 6L lavadas previamente con ácido. Se añadió una solución de $NaH_2PO_4.H_2O$ (1 M) hasta obtener una concentración de 0,05, 0,10 y 1,00 $\mu mol \cdot l^{-1}$, en los distintos grupos de botellas. Paralelamente, se dispusieron 3 botellas sin tratamiento y en las mismas condiciones, como control del experimento. Una vez añadido el fosfato, las botellas fueron sumergidas hasta una profundidad de 1 m. Las mismas eran sacadas del agua dada 12 h para colectar una muestra para el análisis de Chla, procurando no extraer más del 10% del volumen total al final del experimento. Por último, se estimó la APA mediante el método de células marcadas con ELF descrito anteriormente.

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Capítulo 1

Variabilidad biogeoquímica episódica en un estuario Mediterráneo de flujo bajo. / Episodic biogeochemical variability in a low-flow Mediterranean estuary.

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Abstract

To investigate to what extent episodic physical processes regulate nutrient availability and phytoplankton assemblages of the Mahon estuary (Minorca Island) we carried out an intensive field study during 2010-2011. During the study period, environmental conditions spanned from intense stratification to a continuous mixing and from lack of riverine inflow to intense runoff. Our data reveals a sequence of biogeochemical states of the estuary that result from the interplay between runoff, other non-periodic forcings (winds, sea-level oscillations) and variations in water renewal. Seasonal runoff was revealed as a major driver of winter circulation and of the influx of inorganic nutrients, in particular nitrate. However, because of the combination between runoff and flushing time, the effects of floodwater events on phytoplankton are short-lived (days). Conversely, during summer, when freshwater influx declines, water renewal relies on pulsed atmospheric forcing that may be of local or remote origin. As depicted from the low nitrate concentrations (<1µM) and enhanced ammonium (>1µM), this change in circulation and external loads carries nutrient assimilation within the estuary head and forces the use of remnant nutrients through regenerating pathways to sustain an enhanced phytoplankton biomass at the lower estuary. Episodic variability represented between 52 and 65% of the annual

chlorophyll variance. Despite the fact that episodic pulses represented intense departures from base biogeochemical state of the estuary, at time scale larger than weeks, the phytoplankton community composition and dynamics was largely regulated by the integrated effect of these episodes and other environmental drivers associated with seasonality rather than by individual storm events only. Our results suggest that even though the system presents good recovery capacity to individual storm episodes, it may be more vulnerable to increased nutrient fluxes during summer, as well as to changes in episode timing and frequency.

Highlights

- We analyze the physical and biogeochemical functioning of a microtidal estuary.
- Seasonality is a major driver of the environmental and biological conditions in the estuary but a variety of atmospherically forced episodic processes confer its peculiar Mediterranean character.
- Event driven runoff forces the winter estuarine circulation and controls both the influx of nutrients and phytoplankton biomass within the estuary.
- During summer, circulation is highly variable and event-related favoring slow salinity increase and biomass accumulation.
- Phytoplankton community dynamics is regulated by the integrated effect of the episodes rather than by individual events.
- The estuary reveals to be more resilient to episodic disturbances than anticipated but higher vulnerability to change is expected during the summer stratified season.

1.1 Introduction

Ecological variability patterns in coastal systems are typically characterized by responses to periodical physical and chemical forcing, induced by both low frequency periodic forcings (e.g. seasonality) and by tides and other short-term oscillations (e.g. wind mixing, solar irradiance, etc. e.g. Blauw et al. 2012). Superimposed on these periodic variations are other aperiodic or episodic disturbances which are generally mediated by changes in the atmospheric forcing (e.g. Chen et al. 2010). The effects of this shorter-term variability are often considered as 'noise' blurring the relevant ecological patterns. In some coastal areas, however, particularly in those with small tidal range, the event-scale physical variability can be a major regulator of the biogeochemical fluxes, inducing profound impact on the structure and functioning of their respective coastal ecosystems (e.g. Gillanders and Kingsford, 2002).

Many regions of the Mediterranean coasts present this duality between seasonal and weather driven ecological variations (e.g. Arin et al. 2013; Romero et al. 2014). In open waters, seasonality is characterized by a strong contrast between the stratified oligotrophic conditions occurring in summer and the more productive winter mixed conditions (Siokou-Frangoul et al. 2010). Conversely, fueled by the high nutrient availability from anthropogenic sources, in nearshore waters maximum production often occurs in summer (e.g. Garcés and Camp, 2012). In the absence of significant tides, coastal circulation in the Mediterranean is highly conditioned by changes in wind forcing, and thus coastal water renewal responds to these variations (e.g. Jordi et al. 2011). In these waters, the canonical seasonal pattern can be markedly disturbed by episodic variability of meteorological origin, manifested as sudden and intense wind pulses and/or intense rain episodes lasting for few hours to days interrupting the overall calm conditions. Sea-level variations and runoff are also major regulators of coastal-ocean exchanges. Most notably, irregularity and often very stormy precipitation pattern result in null or small base freshwater flow during long periods that are disrupted by pulses of flash-floods transporting sediments, nutrients, organic matter and other compounds from a watershed to the coast. During these events freshwater discharge to the coast can be multiplied by a factor of ten (Estrela et al. 2001), delivering a significant proportion of the annual loads of inorganic and organic compounds to the coastal zone. The biogeochemical changes associated to these episodic stress events provoke departures from equilibrium states that differ depending on a number of biotic and abiotic factors (geomorphology, ecosystem structure, etc.), giving rise to a variety of ecosystem responses.

Due to their confined nature, and the integrative effects they exert over the input from extensive regions, estuaries, bays and other semi-enclosed coastal areas are particularly responsive to episodic events affecting their terrestrial and oceanic boundaries, and present marked fluctuations in their environmental conditions (Cloern, 1991; Harding, 1994; Paerl, 1997). Estuaries, and other transitional environments, provide habitat for diverse marine communities and natural resources of economic and social importance, and have a functional role as ecotones intervening in the decomposition of organic matter and nutrient cycling (Levin et al. 2001). Ephemeral river discharges, together with strong evaporation and weak tidal mixing, result in complex estuarine flow patterns under the Mediterranean climate, including quasi-neutral (stagnant) flow conditions and water circulation reversals (Hearn, 1998; Largier et al. 1997). These flow variations strongly determine the exchange with the ocean, thereby affecting water properties. Moreover, connections between benthic and pelagic environments may be either reinforced during enhanced energy periods, by increasing material processing and nutrient cycling, or uncoupled under low forcing situations (e.g. Ubertini et al. 2012). These variations have consequences in the biogeochemical role of estuarine systems in response to the changing forcings. For example, they can act as preferential transference conduits of organic matter and nutrients that subsidize the adjacent coastal areas, as biochemical filters that retain certain elements, or as self-contained systems uncoupled from the coastal ocean. Nutrient availability can also cause shifts in function through changes to primary production that transfer through the food change to modify the ecosystem structure (Borum and Sand-Jensen, 1996; Nielsen and Richardson, 1996). Phytoplankton response to nutrient enrichment, not only depends on the load but also on the stoichiometric ratios, timing, rate and pattern of delivery (e.g. Howarth, 1988).

The increasing anthrophized nature of the coastal areas, and the consequent modification of the biology and chemistry of the seawater and sediments that it involves, may have altered the resilience of coastal systems to environmental variability. This has noticeable consequences in the biogeochemical cycling of nutrients and in the role of coastal areas as buffer zones between terrestrial and marine systems. Although it is a global issue, anthropogenically enhanced delivery of nutrients and other elements to the coast may be particularly critical in Mediterranean oligotrophic areas, where inputs from terrestrial systems account for a larger fraction of the coastal budgets, and where even small disturbances can produce notable effects. However, while the importance of episodic disturbances in the regulation of ecosystem biogeochemistry has been demonstrated in areas of major exchanges, such as large rivers and estuary areas in humid climates (Yeager et al. 2005; Gobler et al. 2005; Rudeck et al. 1991), their effects on the Mediterranean coastal systems have been given less consideration (e.g. Lipizer et al. 2012; Nicolau et al. 2006; Spatharis et al. 2007).

Semi-enclosed water bodies fed by small fluvial drainage basin environments are common systems in low tidal range, semi-arid Mediterranean regions (Lichter et al. 2011). Most of the studies carried out to date in the Mediterranean are centered on the consequences of flash-flood events (e.g. Guizien et al. 2007). Evidence shows that despite their low magnitude and transient nature, episodic flash-flood events constitute a large proportion of the exchanges along the Mediterranean coasts (Tzoraki and Nikolaidis 2007). A central question to understand the functioning and the response to anthrophization of Mediterranean coastal areas is how and to what extent the ecosystem is regulated by episodic events, and what are the mechanisms that alter the pathways of major nutrients entering the system.

The present work focuses on the nature and relative importance of non-extremal short-term variation in the biogeochemical dynamics of a low-flow Mediterranean estuary, Mahon. In temperate coastal zones, episodic meteorological forcing may have a strong impact on biogeochemical properties and planktonic ecosystem dynamics (Boldrin et al. 2009; Guadayol et al. 2009; Malej et al. 1997). Moreover, projections suggest that the Mediterranean is a primary hotspot of climate change (Giorgi 2006) and the distribution frequency of intense atmospheric episodes such as precipitation events is expected to vary in this region (Alpert et al. 2002; Giorgi and Lionello 2008). This episodic variability has an impact on some key ecosystem features such as estuary–ocean exchange, which may influence the ecosystem behavior and water quality. Knowledge on the overall influence of these disturbances on the functioning of these ecosystems is critical to our understanding of their vulnerability to ongoing climate and anthropogenic changes.

1.2 Material and methods

1.2.1. Site description

Mahon is an elongated estuary (~ 5.5 km long) located in the Island of Minorca (Balearic Islands), in the western Mediterranean Basin (Fig. 1.1). The peculiarly narrow and deep morphology (max. depth 29 m) is conditioned by a W-E running geological fault over which the harbor settles. The main axis of the basin veers slightly (30°) to the south in its outer middle section (from Illa del Rey). The inner sub-basin is shallower (max. depth of 12m), and connected to an ungauged stream that runs through a small agricultural area. Flow is negligible except during major storm events.

A watershed with an approximate area of 27.9 km2, 18% of it corresponding to an impervious substrate, drains mainly in the head of the estuary. Owing to the impervious land surface, even minor storms may lead to an increased runoff and small peak flow events. The mouth of the harbor is very narrow (~300m) and shoals to ~14 m, thereby constraining the exchange with offshore waters.

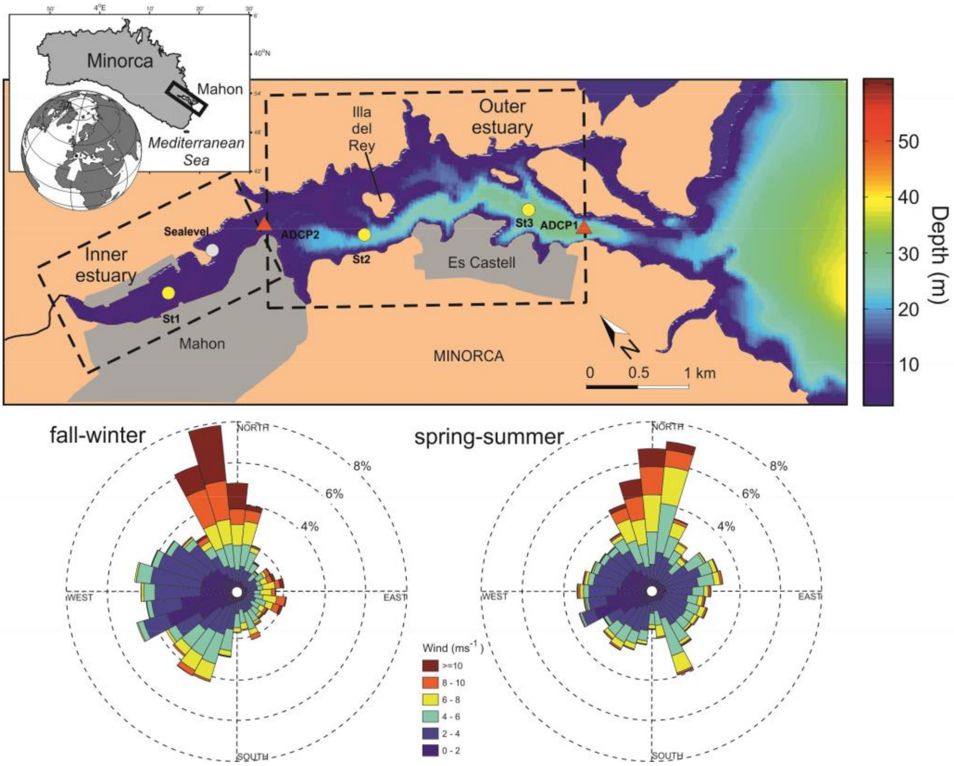

Figure 1.1 (Top) Map and bathymetry (m) of Mahon estuary. Dots represent the sampling stations occupied during the various surveys. Time series stations are indicate with yellow dots. Red triangles and cyan dots indicate the position of ADCPs, sealevel and CT recorders. (Bottom) Seasonal wind rose diagrams based on 10 minute wind data recorded at Mahon during the present study.

Climate in the area is characterized by a strong seasonality, mild wet winters, and warm to hot dry summers. The island is affected by strong Tramontana events, a katabatic (downslope) wind blowing from the north that cools the climate. They occur with greatest frequency in the fall and spring, being less common and weaker in midwinter (January) and summer (see Fig. 1.1a). A progressive shift to easterly winds, together with a general weakening of wind intensity, is related to the onset of a breeze regime occurring from mid-April to the end of summer. In early fall, and throughout the winter, southeasterly winds blowing towards the estuary entrance also become frequent. Wind from this direction precedes the passage of low pressure systems from the Iberian Peninsula that is associated with heavy rain episodes (Romero et al. 1998). These SW events do not generally expand for more than two days because of the eastward displacement of the depressions (e.g. Doswell et al. 1997; Ramos et al. 2014).

Precipitation is strongly seasonal, with the rainfall season beginning between late summer and early fall. Precipitation is rare from June to late August, whereas records from the National Meteorological Agency (AEMET) reveal high frequency in moderate rainfall events (>10 mm day-1) from September to December and in spring (Fig. S1.1b). Indeed, precipitation during fall is typically a major contributor to the annual precipitation (mean 599±99 mm for the period 2000-2015), with 40±11% of precipitation occurring between October and December. Coastal water temperature ranges between 12 and 27°C throughout the year. Sea-level variations are governed by mixed semidiurnal astronomical tides (amplitude <0.25m), and similarly important atmospherically forced sea-level variations. Since astronomical tidal range is low relative to the depth of the estuary the tidal prism only represents 0.5% of the total volume of the basin.

Two urban areas settle along the southern coast, Mahon and Es Castell, with 30,000 residents and 8,000 residents, respectively, but total population (residents+vistors) peaks at summer during the high tourism season (2.5 fold winter population). The wastewater effluents of these urban areas are collected through a sewer that discharges offshore, yet, effluents from some buildings in the port area may seep to the estuary representing a minor, but continuous, eutrophication source. These discharges enrich the water at the head of the estuary with nitrate, and less so with phosphate (Martinez-Soto et al. 2015). Storm-water from impervious urban surfaces flows directly into the bay following the steep coastal topography.

1.2.2. Environmental data

Between July 2010 and July 2011, continuous near-surface measurements of temperature and salinity were obtained at 1 minute intervals with a SBE-CT installed at the shoreline some 0.6m below the sea surface (CT in Figure 1.1). Current velocity profiles were obtained at two bottleneck points using bottom-mounted ADCPs (1000 kHz Nortek Aquadopp profiler), deployed at depths of 25 (ADCP1) and 8 m (ADCP2, see Fig. 1.1). Currents were measured at 1m vertical intervals with the first level 1m above the bottom. In accordance with the other sampling devices, the temporal sampling interval was also set to 1 min in both ADCPs. Forty-three temperature and salinity vertical profiles were obtained at each of the three sampling stations (St1, St2 and St3; Fig. 1.1) at variable intervals spanning from 7 to 10 days, using a YSI CastAway CTD that was deployed slowly to obtain good vertical resolution. The depths at each station were: 6m at St1, 21m at St2 and 27m at St3. Underwater irradiance was measured during four seasonal surveys using a Li-Cor spherical sensor and the diffuse attenuation coefficient (k) was calculated from least-square fit of the log transformed data.

Sea-level variations were registered at 5 min intervals by a MIROS microwave radar recorder located at the inner basin (Fig. 1.1). This instrument is part of the sea-level measuring net REDMAR maintained by the National Port Authority 'Puertos del Estado'. Continuous wavelet transforms (CWTs) were used to determine the degree of non-stationary oscillations present in the sea-level time series. The application of the CWTs is an effective method of analyzing and synthesizing the variable structure of a signal over time, and provides a means of localizing events of interest at their exact temporal location (Torrence and Compo 1998). We used a Morlet wavelet, commonly used in studies of geophysical processes.

To analyze the mass exchange between the estuary and coastal waters, the current flow vector time series for each moored ADCP location was decomposed into an along (u) and cross-channel (v) component. Since our interest was the subtidal variability in the estuary and, in particular, the identification of event-related anomalies, the wind, sea-level, and current data sets were low-pass filtered using a 33h filter to remove fluctuations with diurnal or higher frequencies (Beardsley and Rosenfeld, 1983). We concentrated our analysis on the study of two periods; one covering the summer until the first autumn rainfall episode (period 1, summer: 2010/07/15 to 2010/10/15), and the other spanning from December to March (period 2, winter: 2010/12/15 to 2011/3/15). We selected these periods because they represent the two seasonal extremes in water column stratification while including relevant meteorological events (i.e. rainfall and wind). Empirical orthogonal function (EOF) analysis was applied to the along estuary component (u) of the current velocity to obtain a representation of the main modes of variability. The EOF analysis decomposes spatial-temporal variations of a geophysical field into combination of orthogonal spatial patterns with corresponding principal components in a linear fashion (Lorenz, 1956).

Meteorological data was obtained from the station of Mahon Port Authority located in the mouth of the estuary. Additional meteorological data from Mahon Airport located

3.5 km to the south of the study area were obtained from the Spanish Meteorological Agency (AEMET). We used wind and atmospheric pressure data from the Port Authority because they reflect better the conditions in the coast. However, precipitation measurements of this station were unreliable and, therefore, rainfall data from the Airport was used. Spectral coherence was estimated using sea-level and along-axis current with the wind rotary components as a complex time series (Gonella, 1972; Mooers, 1973). A Hanning window of 1440 points (10 days) with half window overlapping was applied for all spectral computations, resulting in at least 12 degrees of freedom (dof) for the shorter (winter) time series. Daily flushing time (τf =volume/(cross sectional area*u)) for the inner and outer domains of Mahon harbor was estimated from hourly mass exchange estimations through control bottle-neck sections at ADCP1 and ADCP2, assuming that flow was homogenous at each depth level. Since the ADCP data does not reach the surface, near-surface current intensities (i.e. 1 m) were linearly extrapolated from the levels below. The highest value of the inflow-outflow at each section was used to calculate the exchange between the inner and outer estuary or between the estuary and the coastal ocean (see Fig. 1.1). The calculated flushing time values represent an upper-end estimation of the water renewal in the estuary, which is assumed to be lower since a large part of the flux is produced by oscillatory motions that do not produce significant exchange.

1.2.3. *Sample collection and analysis*

Discrete seawater sampling was carried out simultaneously to CTD vertical profiles. Due to logistic limitations, runoff episodes were characterized by the conditions in the estuary within three days after each major rainfall event. At each sampling station (St1, St2 and S3), triplicate surface water samples (13 ml) were collected by hand in acid-washed polyethylene tubes for nutrient analysis. Additionally, seawater samples were obtained at 6 depths at St3 on 2010/10/21, 2011/03/11 and 2011/06/27. A 2.5L Niskin bottle was used to obtain these samples. Nutrient samples were pre-filtered through GF/F fiberglass filters (Whatman Ltd.) and subsequently frozen until analysis. Concentrations of dissolved inorganic nitrogen (DIN: NO_3^-+NO_2^-+NH_4^+), phosphate (PO_4^{3-}) and soluble reactive silicates (Si) were measured with an Alliance Futura autoanalyzer following Grasshoff et al. (1983). Total dissolved P was estimated by pre-oxidizing the sample with an alkaline persulfate digestion that hydrolyzes all forms of inorganic and organic P to orthophosphate. Dissolved organic phosphorus (DOP) was estimated as a difference between TP and DIP. The accuracy of the analysis was established using Coastal Seawater Reference Material for Nutrients (MOOS-1, NRC-CNRC), resulting in 97%, 93%, 98%, and 118% for PO_4^{3-}, NO_3^-, NO_2^-, and SiO2, respectively. The Limit of Detection (LOD), calculated as three times the standard deviation of subsequent blank measurements, was PO_4^{3-}: 0.04 µM, NO_3^-: 0.001 µM, NO_2^-: 0.001 µM, NH_4^+: 0.03 µM and SiO_2: 0.02 µM and the precision <2 % RSD (relative standard deviation) for all nutrients.

Chlorophyll (Chl) concentrations were measured by fluorometric analysis of extracted pigments. About 120 ml were filtered through glass-fiber filters (GF/F-Whatman) and stored frozen (Parsons et al. 1984). Pigments were extracted in 90% acetone and stored in the dark at 4°C overnight. The fluorescence of the extracts was measured with a Turner Designs fluorometer calibrated with pure Chl-a (Sigma Co.). The chlorophyll time-series was low-pass filtered to separate seasonality from the higher frequency signal at each station.

Samples were collected for the identification and quantification of microplankton assemblages and were maintained in Lugol-iodine solution, sedimented (24 h), and a suitable area quantified using a Leica-Leitz DM-IL inverted microscope (Andersen and Throndsen 2003).

1.3 Results

1.3.1. Estuarine dynamics

Meteorological observations at Mahon indicate that winds in this region present seasonal differences, being highly variable in speed and direction. As shown in Figure 1.1, strongest winds (i.e. >8 m s^{-1}), from which the estuary is protected are predominately from the north and reflect Tramontana wind conditions. Precipitation during the study period (752 mm) was above average (Fig. S1.1b). The wet season started relatively late, but rainfall events associated with the passage of atmospheric depression were noticeably intense, particularly between October and December (Fig. 1.2). Changes in near surface salinity reflected the freshwater flow along the estuary in response to these rainfall episodes, and significant negative correlation ($r=0.74$, $p<0.001$) was obtained between these surface water salinity variations and moderate rainfall events (accumulated precipitation >15mm/day). Intense precipitation (>15mm/day) occurred on 15 days clustered on 8 major events, associated with the passage of atmospheric depressions affected the estuary between September and April 2010. Mahon received significant inputs of freshwater during these events, but recovered quickly (5±2.2 days) leaving only a variable background salinity decline of 0.15 to 0.51 units. Mean surface salinity during the sampling was 37.5±0.44 in the inner estuary. Between July and September, salinities in the inner basin exceeded 38.0 revealing scarce freshwater influence. These high salinity conditions were generated at the very slow 0.003 units day^{-1} over the period of a month, presumably by the combined effect of oceanic water intrusion and evaporation, and finished abruptly with the first rainfall event at early fall.

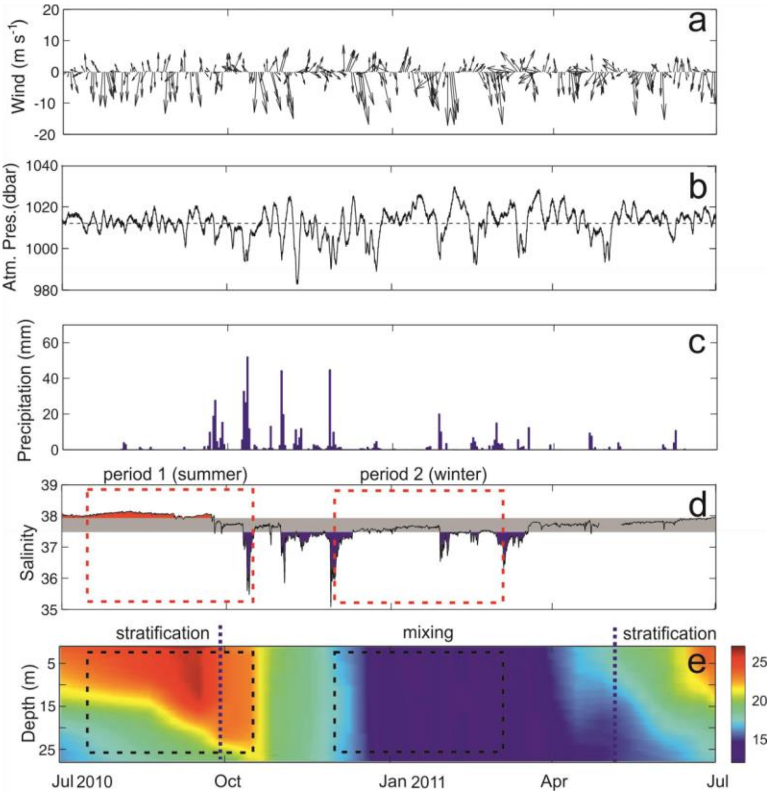

Figure 1.2 Atmospheric conditions at Mahon during the survey period. a) mean diel wind vector; b) atmospheric pressure; c) accumulated daily precipitation. d) continuous record of near surface salinity at CT and e) water column temperature (oC). Blue and red areas in the salinity figure indicate values below and above the typical range (gray band). Dashed boxes indicate the summer and winter period analyzed in detail. Blue dashed lines mark the approximate limits of the stratified and non-stratified periods.

The first rainfall event did not produce a marked salinity variation, most probably because land was dry and warm, and runoff was scarce. Subsequent storms occurring during the fall were most intense, producing a decline in salinity of up to 2.2. From late fall to late spring temperature and salinity profiles showed minor differences between surface and bottom waters, but during summer conditions changed remarkably and the water column presented intense thermal stratification with a thermocline located at 16.3±1.0m isolating the deepest waters of the harbor. Temperature difference between the upper and lower layer at the beginning of this period exceeded 10°C but temperature progressively equilibrated and thermocline deepened by the end of summer.

Figure 1.3 displays the decomposition of sea-level variations into tidal and non-tidal components, illustrating the importance of non-tidal low frequency variations. Tide was determined by semi diurnal and tidal constituents (M2, K1, S2, O1 Table S1) whereas high frequency sea-level oscillations were caused by internal seiching motions peaking at 37, 14 and 8 min. Most notably, subtidal sea-level variations exceeding the tidal range were frequent throughout the year, particularly at the end of summer and along the winter. The CWT analysis of these variations highlights the relevancy of the synoptic meteorological scales (i.e. 2 to 5 days) in these sea-level variations (Fig 1.3c). Indeed, a correspondence between some rainfall, wind and atmospheric pressure variations with the energy enhancement at these frequencies can be inferred.

Currents exhibited predominately semidiurnal fluctuations, but an important subtidal variation was also observed, mostly in an E-W direction being caused by the orientation of the estuary. The EOF decomposition of the subtidal axial-current variance for the stratified (summer) and non-stratified period (winter) is displayed in Figure 1.4. During stratified conditions currents below the thermocline (~16 m) were extremely low (<0.01 m/s) and circulation was highly variable and event-related. Since flow in the deepest layer was negligible, circulation was restricted to two layers above the thermocline. The vertical extension of the deep layer increased as the thermocline deepened after mid-September. The first EOF, capturing 65% of the variability, reveals the pulsed variation in current intensity together with flow reversals. Although the intensification of some of these flow variations can be associated with some of the observed axial wind patterns (Fig. 1.4c), the interpretation is not straightforward, and remote processes (i.e. shelf dynamics) could have a strong influence. This is not the case of the second EOF (18% of the variability) which appears to be associated with local rainfall events.

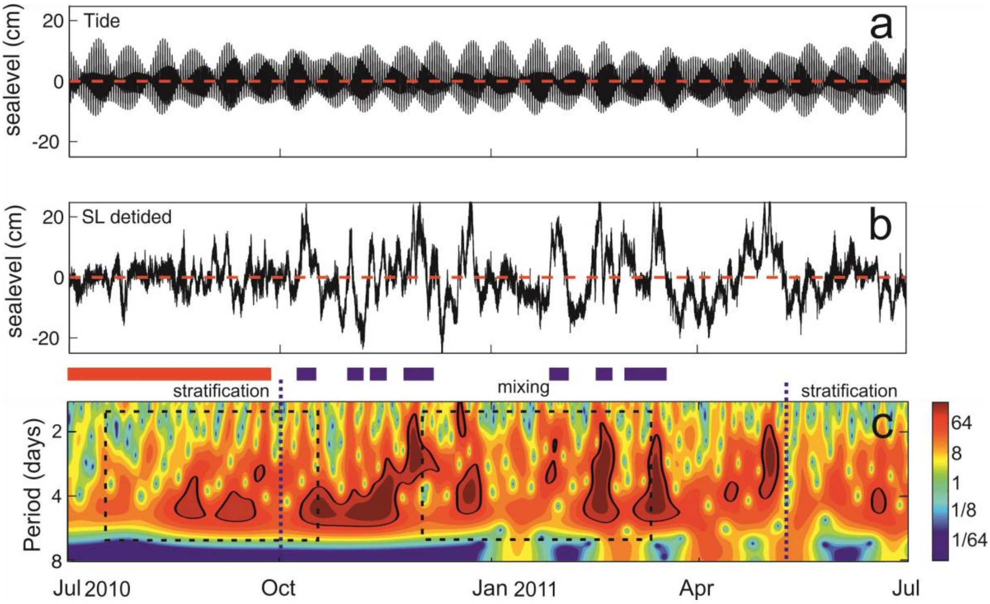

Figure 1.3 Sea-level variations at Mahon from July 2010 to July 2011. a) Astronomical component and b) residual sea-level variations. c) continuous wavelet transform (CWT) power spectrum of the sea-level oscillations. The thick black contours indicate the 95% confidence level. Dashed boxes indicate the summer and winter period analyzed in detail. Blue dashed lines mark the approximate limits of the stratified and non-stratified periods. Red and blue stripes at top indicate periods of positively (red, above typical values) and negatively (blue, below typical values) altered salinity concentrations.

During the second period analyzed (winter), circulation was mainly estuarine and forced by freshwater flow. It is nevertheless striking that circulation rapidly reversed when freshwater flow ceased. The first EOF, representing 76% of the variability, captures this two layer estuarine circulation. The second EOF mode corresponds to more episodic forcing producing disturbances at the interface and occasionally provoking three layered circulation; however, the coherence with local winds is in this case weaker. Current flow at in the inner basin was consistently shown to be very weak (i.e. <0.06 m/s) and generally offshore, causing a surface and bottom compensatory flow.

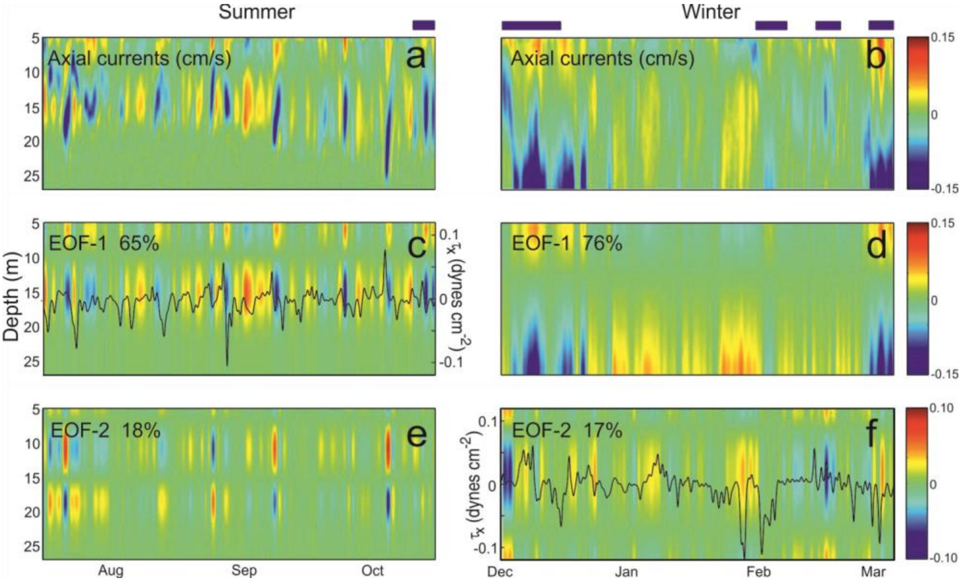

Figure 1.4 Filtered (33h) current record at ADCP1 and first 2 EOF modes during summer stratified (a, c, e) and winter non-stratified conditions (b, d, f). The axial component of wind-stress has been overlaid of panels c and f. Blue stripes at top indicate periods of lowered salinity concentrations.

is rather difficult to disentangle the mechanisms and relative importance through which atmospheric forcing drives circulation in the estuary because, in most of the cases, several processes act simultaneously. However, the importance of some of these processes can be surmised from cross-spectral analyses. For example, as displayed in Figure 1.5, the spectral coherence between the clockwise wind and sea-level displays peaks at frequencies of some 56 and 24 h. These frequencies agree well with those of island trapped waves (ITWs) in the area, as described by Jordi et al. (2009). Both ITWs and breeze combine in the 24h period, which is more energetic in summer, when breeze becomes relevant. The 24h peak is also important in driving the flow between the inner and outer basin (at ADCP2) during summer, and at the thermocline depth at the estuary mouth (ADCP1). Conversely, the 56h period seems dominant at this site during winter.

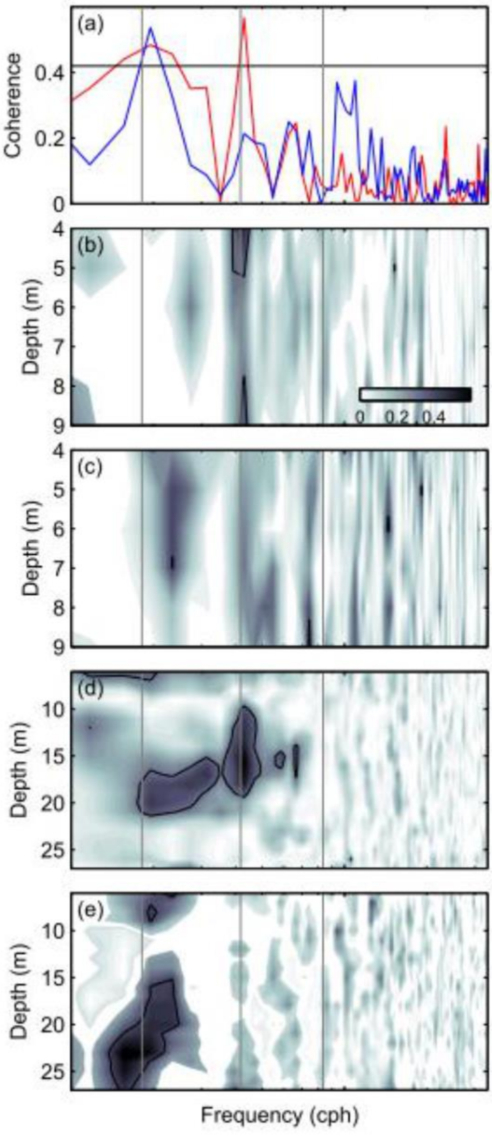

Figure 1.5 Spectral coherence between clockwise wind and (a) summer (period 1, red line) and winter (period 2, blue line) sea level, (b) summer along-axis current at ADCP2, (c) winter along-axis current at ADCP2, (d) summer along-axis current at ADCP1, (e) winter along-axis current at ADCP1. Vertical grey lines are periods of 56.9, 24.1, and 12.4 h. Black lines represent the 95% confidence limit.

In terms of the observed flow, τf ranged from few days to more than three weeks (Fig 1.6). The higher τf values (i.e. τf >15 days) reveal quasi-stagnant situations between flow reversals that do not extend for more than a day. During winter, these transitions presented lower exchange than in summer (τf>23 days), possibly due to the absence of thermal winds. Conversely, reduced renewal events (i.e. τf from 6 to 15 days) were more frequent in summer when currents regularly reversed. Despite these differences mean τf was only slightly higher in summer than in winter and differences were not significant (9.4±5 and 8.1±5, p>0.05).

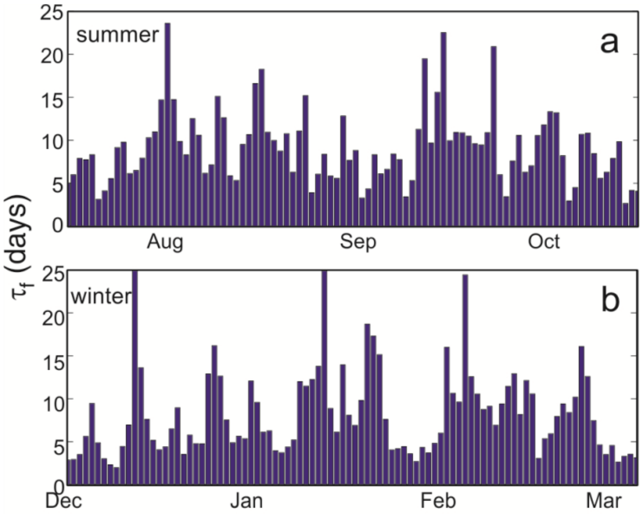

Figure 1.6 Daily variation of τf (days) based on data from ADCP1corresponding to the summer and winter periods.

1.3.2. Macronutrient variability

Overall, the Mahon estuary was enriched in dissolved inorganic nitrogen and less so in Si with respect to open coastal waters. As shown in Table 1.1, an inshore-offshore gradient was observed in most nutrients levels revealing that land sources are a major regulator of the biogeochemistry. Also, as many coastal areas in Balearic Islands (e.g. Basterretxea et al. 2010), the estuary is deficient in inorganic P particularly in its inner basin, where N:P ratios exceed 100. Mean DIN and DIP concentrations at the sampling points revealed these spatial differences and, also, the seasonal pattern (Table 1.1). N and Si increased in the fall and spring during the periods of reduced salinity, peaking during the major rainfall events; whereas P mainly increased during the heavy rains in fall and only marginally in spring (Fig. 1.7). An exception was ammonium which presented marked seasonality, being the dominant nitrogen form in summer and declining to marginal levels when the water column was mixed (<0.5 µM). Average phosphate concentrations were low, but mean concentrations doubled in the fall when runoff was more permanent (Table 1.1). DOP was consistently higher (mean 0.23±0.09 µM) and, therefore, DIP only comprised some 28±18% of total phosphorus. However, rainfall events significantly varied the relative amount of DIP to TP (Figure 1.7c).

Table 1.1 Seasonal changes of near surface nutrient and chlorophyll concentrations in the three sampling stations.

Season	Station	Nitrate [uM]		Ammonium [µM]		Phosphate [µM]		Silicate [uM]		Chl [mg m-3]	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Summer	St1	2.26	0.94-4.62	3.79	1.00-7.23	0.06	0.04-0.14	1.64	0.43-2.76	4.92	2.84-9.46
	St2	0.29	0.06-0.58	2.73	1.99-4.22	0.06	0.04-0.15	1.16	0.48-3.50	4.50	2.20-5.73
	St3	0.06	0.01-0.13	1.95	1.00-2.97	0.05	0.04-0.18	0.63	0.27-1.10	2.29	0.99-4.48
Fall	St1	11.04	2.56-37.32	1.90	0.01-6.66	0.26	0.04-1.08	3.86	1.20-11.65	2.15	1.11-3.72
	St2	3.87	1.06-13.83	1.17	0.01-3.92	0.11	0.04-0.35	1.55	0.14-6.27	4.18	1.45-12.71
	St3	1.58	0.18-6.79	1.11	0.01-3.64	0.11	0.04-0.40	0.89	0.30-1.97	3.26	1.40-9.34
Winter	St1	6.78	3.58-12.81	0.90	0.04-5.62	0.05	0.04-0.11	2.41	1.07-6.29	2.44	1.35-3.79
	St2	2.57	1.41-3.69	0.18	0.01-0.81	0.05	0.04-0.07	1.03	0.14-2.80	2.28	0.74-3.12
	St3	1.16	0.35-1.92	0.12	0.01-0.48	0.05	0.04-0.08	0.68	0.12-1.32	2.01	0.86-2.62
Spring	St1	2.93	0.83-4.90	0.46	0.07-1.23	0.05	0.04-0.13	1.68	0.38-2.96	2.59	1.47-5.53
	St2	0.51	0.01-1.64	0.27	0.05-0.83	0.04	0.04-0.06	0.82	0.14-1.76	2.06	0.96-3.37
	St3	0.29	0.03-1.27	0.31	0.08-0.76	0.04	0.04-0.08	0.61	0.25-1.36	1.07	0.71-1.55

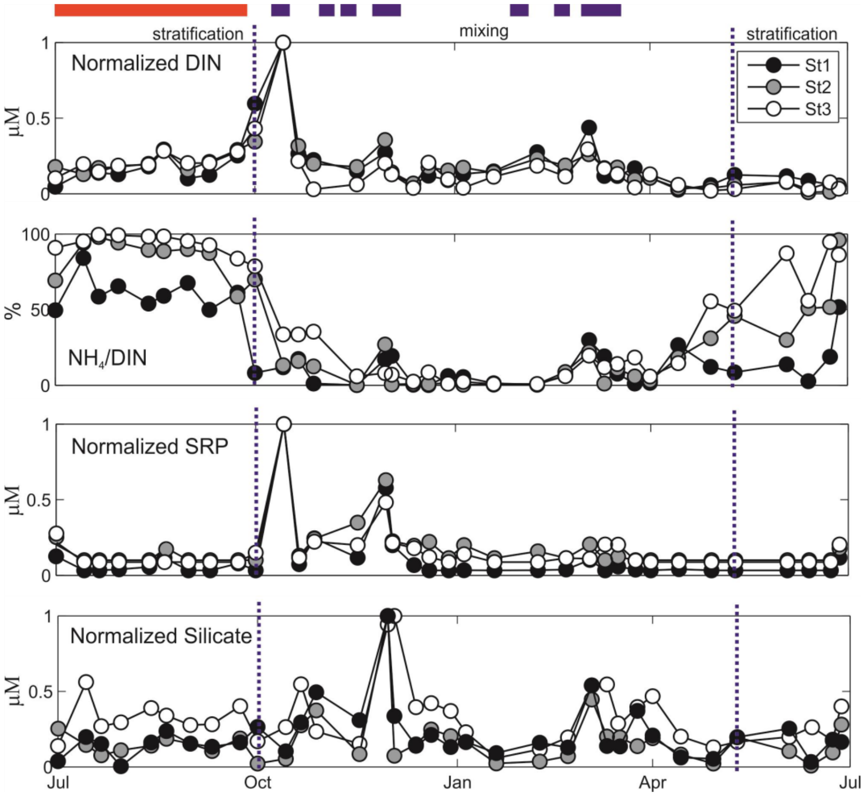

Figure 1.7 Temporal variation of nutrients at Mahon. Concentrations have been normalized to the maximum value for comparison reasons. Red and blue stripes indicate periods of positively (red, above typical values) and negatively (blue, below typical values) altered salinity concentrations.

The vertical profiles shown in Figure 1.8 reveal the water column changes and associated nutrient and Chl variations occurring in the estuary. The water column was thermally stratified in June and the thermocline separated an upper layer with relatively low nutrients from a deeper layer where nitrate and silicate were more abundant, reaching concentrations above 3.0 µM near the bottom. Ammonium represented 78% of DIN in this lower layer during this season. These vertical hydrographic and nutrient differences rapidly homogenized October. During winter, nitrate and silicate were highest at surface, were a lower salinity layer was present. This is consistent with runoff being a main source of nitrate and silicate. DIP was also enhanced in this lower salinity layer but the increase was very weak (0.1 µM) at the sampling point (St 3).

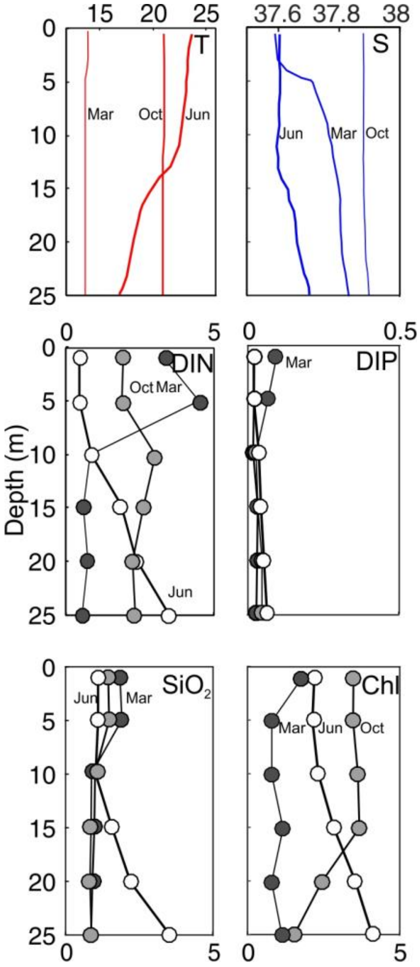

Figure 1.8 Vertical profiles of density temperature (°C), salinity, DIN (µM), DIP (µM), silicate (µM) and chlorophyll (mg m-3) at St3 during October 2101, March 2011 and June 2011

Figure 1.9 shows the differences in the nutrient molar ratios along the estuary. Because of the persistently high nitrate and silicate concentrations at St1, the inner sub-basin was P-limited (N:P > 16) for most of the time. This is consistent with results based on alkaline phosphatase activity obtained by Martinez-Soto et al (2015) at this site. N-limitation was marginal and some episodic increases in P, while reducing P-limitation, drove the system towards Si-deficiency. P-limitation was also the most common state at St2 and St3, but N:P ratios were reduced in the offshore direction whereas Si+N co-limitation became more frequent.

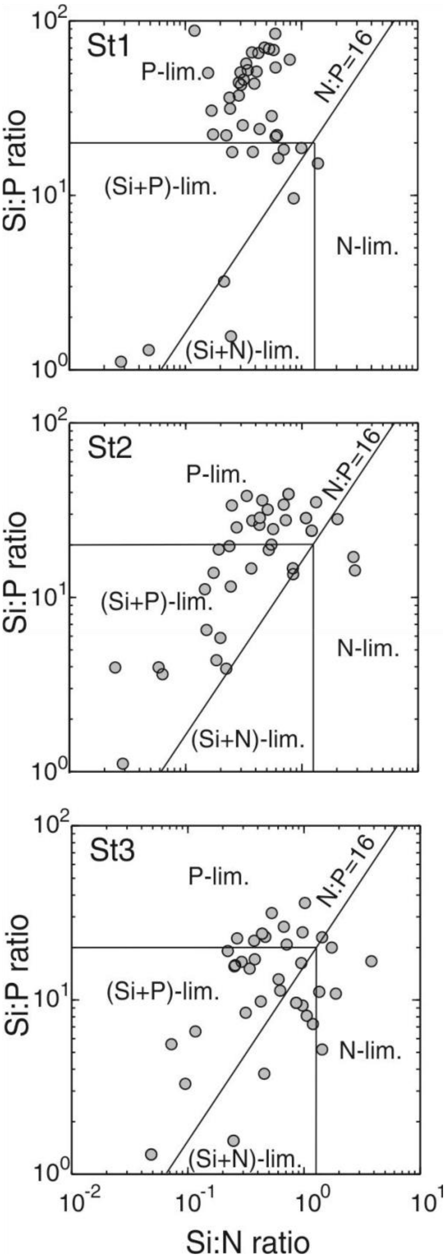

Figure 1.9 Molar ratios of dissolved inorganic nutrients at the three sampling points in Mahon estuary (St1, St2 and St3). The lines separate four theoretical zones representing areas with different nutrient limitations according to canonical Redfield ratios.

1.3.3. Runoff episodes and associated loads

Generally, inorganic nutrients were very responsive to runoff events, but there was not a good correlation between the salinity decline (ΔS) and the nutrient concentrations in the estuary. The influence of runoff was particularly noticeable in the case of DIN and Si, but less so in the case of P. Indeed, differences in DIN and Si between St1 and St2 correlated well ($r=0.83$, $p<0.01$) except when DIN exceeded 6 µM during the rainfall episodes in fall. In these events the nutrients ratios were substantially altered towards an excess of DIN. This was consistent with the elevated nitrate concentrations measured in the torrent (133-150 µM), which is presumably enriched by agricultural loads and decaying plant matter in the lower section of the watershed. Phosphorus was only significantly enhanced during the most intense rains in the fall but otherwise it was present in concentrations at the limit of detection (0.04 µM). As a consequence, runoff had the potential of increasing the already existing phosphorus limitation.

1.3.4. Biological conditions

Chlorophyll concentration within the estuary varied between 0.74 and 12.7 mg m-3, displaying marked spatial differences. Mean Chl was generally, though not always, enhanced in the inner station and decreased towards the estuary mouth (Table 1.1). Light attenuation coefficient (k, m^{-1}) also varied from 0.3-0.5 m^{-1} in the innermost station (St1) and decreased to <0.25 m^{-1} at St3 and presented reasonable correlation with chlorophyll ($r^2=0.8$), except when runoff was active and k values exceeded 0.4 in the inner harbor, revealing strong influence of riverine dissolved and non-biogenic particulate materials in the light field at St1.

During fall, intense chlorophyll enhancements developed in the middle of the estuary in response to water column mixing and intense rainfall episodes. Seasonality represented between 35 and 48% of the variance being lower at St3 where the estuarine processes are attenuated by the greater influence of offshore waters. Chlorophyll concentration was enhanced throughout the summer and the seasonal (low frequency) variation in biomass peaked at the end of the season (except at St3), with the end of the stratification period (Table 1.1 and Fig. 1.10). This seasonal trend contrasts with the canonical pattern of offshore blooms which occur regionally in mid-winter (January-February, Jordi et al. 2009b). Maximum Chl concentrations at St2 and St3 occurred when the water column mixed at the beginning of October (see also Fig. 1.8). Conversely, St1 seemed more influenced by other processes, possibly of biological nature, producing short-span blooms during summer. Indeed, strong rainfall generally produced a decrease in biomass at this site.

Phytoplankton taxa responded to shifts in the conditions of the estuary rather than to episodic forcings. Motile taxa such as dinoflagellates and coccolithophorids prevailed throughout the year, whereas diatoms (mainly Tahalassionema sp. and Thalasiossira sp.) were only present during early fall when the surface water cooled down and the water column mixed (Fig. 1.10). Low diatom abundance was attributed to the low Si availability, since diatoms require a Si:N ratio of ~1 to grow (Dortch and Whitledge, 1992) and, thus, were not able to compete effectively with species without a Si requirement. Also, it is noteworthy that while similar abundances of dinoflagellates and coccolithophorids were present during spring and through summer, the later were dominant in the fall (>90%), when nutrient concentrations were at their highest.

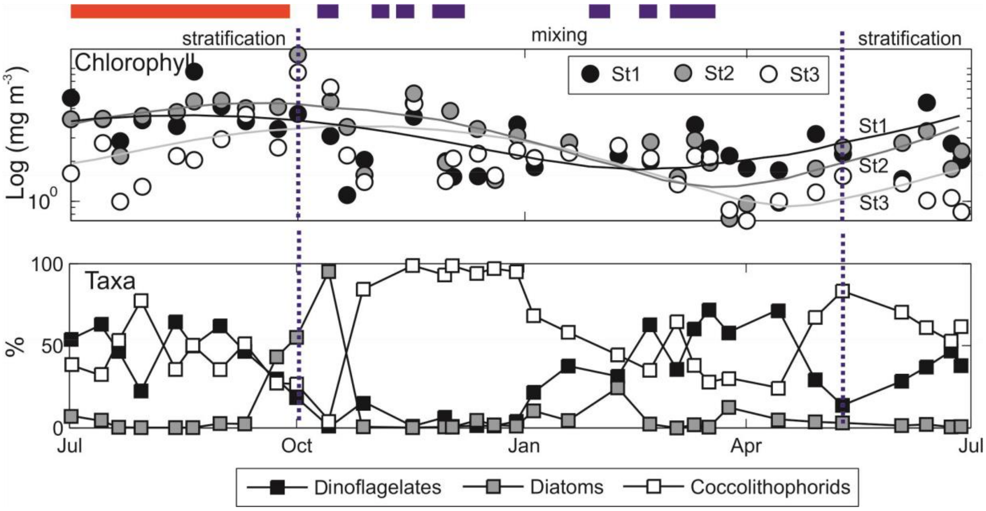

Figure 1.10 Temporal variation of chlorophyll (mg m-3) at Mahon and main microphytoplankton taxa (%) at St2. Chlorophyll is represented in a logarithmic scale. Red and blue stripes indicate periods of positively (red, above typical values) and negatively (blue, below typical values) altered salinity concentrations. Continuous lines represent the low frequency (i.e. seasonal) component of the variability at each station.

1.4 Discussion

1.4.1. Estuarine dynamics

The present study has investigated the dynamics of a low-flow Mediterranean estuary for the purpose of understanding the relevance of episodic forcing. The equilibrium between seasonal processes and episodic events molds the biogeochemistry of Mahon and its various ecological roles. Generally, strong seasonal patterns develop where the governing processes are linked to the annual climate cycle, and large event-scale variability occurs at sites highly enriched with nutrients (Cloern and Jassby, 2010). Mahon responds to both of these paradigms; it displays intense seasonality that determines the general flow patterns in the estuary and overlying remarkable event-scale chemical and biological departures the basal state (defined as the system state during low runoff and weak atmospheric forcing conditions).

The degree of connectivity with the adjacent catchment and the sea, as well as the loads of the associated exchanges, determines the environmental conditions in an estuary. Our data reveals that, despite the daily flushing time being similar in the two periods analyzed, the hydrodynamic functioning of Mahon varies dramatically from summer to winter. It also varies seasonally in the degree of marine influence from fully marine in summer to lowered salinity conditions (salinity <37) later in winter. Summertime dynamics is characterized by negligible runoff, which has profound effects on the circulation pattern of the estuary that relies on oscillatory motions forced by local and remote (i.e. through ITWs) wind forcing. In this sense, the existence of increased wind episodes and their frequency becomes fundamental to the flushing of the estuary. Geyer (1997) analyzed strong influence of local wind in the flushing of two estuaries in Waquoit Bay (USA) that was dependent on their shallow depths and geomorphologic constrains. The case of Mahon is somewhat different because of its greater depth (up to 29m); and yet, the intense summer stratification decouples the surface waters, which are subjected to wind dynamics, from the subsurface layer that remains stagnant (Fig. 4a). This facilitates the response of the estuary to wind forcing which is also favored by the low horizontal density gradient that accentuates the relative influence of wind-stress over the gravitational component. Although perturbed by other effects, the EOF analysis shows this local wind induced modulation in the strength of the estuarine flows, also suggesting flow reversal during the relaxation periods after some episodes (see Fig. 4c).

The relevance of local wind in the flushing of Mediterranean estuaries has been previously acknowledged (e.g. Hearn and Robson 2002); however, less is known on the effects of trapped waves. O'Callaghan et al. (2007) observed that remotely forced shelf waves were important for the circulation and water properties of a microtidal estuary in Western Australia. We also found that oscillatory motions in the range of 1-3 days were an important component of flushing. Nevertheless, it is uncertain to what extent these back and forth motions affect the residence time, and thus the environmental state of the waters in an elongated estuary such as Mahon, particularly in its inner section. Indeed, Rodellas et al. (2015), based on radium isotope techniques, estimated higher mean water ages at Mahon in summer (6.7±1.8 days) than in other seasons (4.2 and 2.3 days). This suggests that despite the comparable τf, water renewal might be, in fact, weakened in summer. Dependence of τf on wind forcing implies that prolonged wind weakening periods may result in reduction in flushing by seawater and consequent enhancement of problems related with nutrient enrichment and associated eutrophication in this season.

Unlike in the summer, wet season dynamics at Mahon is conditioned by rainfall events and, as in most estuaries, freshwater discharge, and gravitational circulation dominate the circulation (e.g. Dyer, 1997). The wavelets analysis revealed narrow coupling between sea-level variations and rainfall events in the period range of 2 to 6 days but, even more important as the rainfall episodes themselves, is the overall effect of runoff on the estuarine circulation. This variation in the importance from short to longer term forcing is evidenced in the comparison between summer and winter flow shown in Figure 1.4. However, because of the small catchment and rainfall irregularity, this type of circulation may dramatically weaken during some periods in winter (i.e. on January) and also presents strong variability. This high degree of stochasticity in the riverine flows makes difficult to obtain generalized patterns of estuarine functioning throughout the winter.

1.4.2. Biogeochemical effects

Two major nutrient sources with their associated temporal variability overlap at Mahon; diffuse urban effluents and eventual runoff episodes. These sources, together with the internal biogeochemical mineralization processes, determine the nutrient balance of the estuary. The wet season is dominated by reduced biological activity (i.e. 43 to 52% of summer phytoplankton biomass), surface water outflow and water column mixing. In contrast, summer is an environmentally vulnerable period at Mahon because, owing to the lack estuarine circulation, the long-term exchange of suspended and dissolved matter is driven by weather stochasticity (i.e. episodic events) and oscillatory motions. Apart from the reduced nutrient loads, the major geochemical variation in this season is the increased NH4 concentration (see Table

1.1), which suggests intensive nitrogen recycling in the estuary and/or decrease of the nitrification rates of diffuse urban effluents. Low inorganic phosphorus concentrations also indicate that internal biogeochemical mineralization and/or organic P sources may be fuelling the phytoplankton standing stock throughout the summer. As a result of the increased biological activity within the estuary during this season, phytoplankton is able to fully assimilate the external nutrient loads (see summer nutrients in Table 1.1). This provokes a marked contrast with the biological conditions offshore, characterized by low chlorophyll values (i.e. Jordi et al 2009b), evidencing a human induced shift in the canonical patterns of the coastal environment. Also, the dominance of dinoflagellates (40 to 60% of total microplankton cells) during this season could respond to their efficiency in the use of regenerated nutrients (Smayda, 1997; Pitcher et al. 1998). Overall, this reflects a change in the estuary role from a transition ecosystem (i.e. sensu Levin et al. 2001) to a self-contained system.

Overlapped on this seasonal trend, runoff episodes suppose large departures from basal nutrient levels exceeding the long term annual nutrient load. In this semi-arid watershed, the nutrient flux during each episode is not linearly related to precipitation intensity and more complex relationships between precipitation and freshwater loads become necessary to explain runoff discharges into the estuary (i.e. duration, storm trajectory across the watershed, soil saturation, etc.). Precipitation events have short-term effect on the biogeochemistry of the estuary, and also a longer term influence that depends on the combined effect of a sequence of storms. Long-term records from AEMET show that between 15 and 30 moderate precipitation days (> 10 mm/day) occur each year at Mahon clustered in 5 to 12 major precipitation events mainly occurring between late summer and late spring (mean 8.5±2 events). This suggests that on average year conditions should not be very different from those observed during the present survey. Only in driest years such as 2000 or 2012, with accumulated precipitations of 419 and 360 mm, winter estuarine circulation could be endangered.

There is profuse evidence of short-lived water quality changes in estuaries in response to storms and floods (Eyre and Ferguson, 2006; Tomasko et al. 2006; Valiela et al. 1998). A main feature of episodic freshwater inflow is its substantial impact on residence time. Suspended particulates, nutrients and biota are flushed out of the estuary during the strong rainfall episodes. During these events the estuary acts mainly as a conduit; although, some physical and biological filtering occurs within the system. Some nutrients remain for sufficient time to stimulate positive phytoplankton responses, and changes in phytoplankton composition following event-scale perturbations have been documented in other estuarine systems when conditions stabilize (e.g. Pinckney et al. 1999; Lomas et al. 2009) but these alterations are usually temporary. Transient responses of the phytoplankton community composition to episodic forcing have been shown in field studies and simulation models revealing tight coupling between the environmental variables, and the phytoplankton response at the time scale of few days (i.e. Nakane et al. 2008). However, as reported for other estuaries (Hall et al. 2013), our results demonstrate a stronger linkage between the phytoplankton community and climatic drivers such as flow, temperature or stratification. The considerable capacity of Mahon to recover from these point disturbances could be related to its relatively large volume that buffers peak events.

Our data also reveals that the timing of runoff events is relevant. For example, runoff episodes occurring at the end of the stratified season present higher biological response than those occurring in winter. This is more evident at St2, in the middle of the estuary which is less influenced by other factors such as increased turbidity, often occurring at St1 or by dilution with offshore waters. This seasonally varying response to increased precipitation has also been observed by Thompson et al. (2015). Similarly, it has been suggested that tropical cyclones which strike temperate estuaries in late fall may have a less severe effect on water quality than do those that occur during warmer summer months (Mallin et al. 2002).

Despite the fact that particular events may not provoke long-term responses in the plankton assemblage composition, their impact can be evaluated by their overall effect. For example, recurrent freshwater discharge may be considered as a disturbance factor that leads to a regular setback of community development, while maintaining the system in an immature state (Stanley 1973). Also, the combined effect of several episodes may produce long term changes in the biochemistry of the estuary. In our case, runoff mainly provides nitrogen and, less so, other elements such as Si. Silica in rivers originates mainly from rock weathering, and direct inputs through urban or industrial wastewater discharge are minor (Garnier et al. 2006; Sferratore et al. 2006). Excess supply of these nutrients represents a fundamental difference in the biogeochemical functioning of the estuary, which is dependent on P availability, with respect to open coastal waters, where inorganic nitrogen is scarce. Phosphorus deficiency does not represent a limiting factor for overall phytoplankton growth, but it triggers the use of alternative P-sources resulting in variations in the competitive advantages of the different phytoplankton species (Martinez-Soto et al. 2015). Indeed, while Mahon is a net exporter of inorganic nitrogen it either scavenges P from nearby coastal waters or relies in the P-supply from human activities either in organic or inorganic forms.

There is a range of climate change related mechanisms through which the estuarine water biogeochemistry is anticipated to be affected, including temperature increase, changes in the hydrological cycle, modification of wind patterns and sea-level rise (Scavia et al. 2002; Rabalais et al. 2009). From these, changes in wind, temperature, and in hydrology seem to be critical for Mahon. As discussed, wind is a major regulator of the water renewal at Mahon, and therefore changes in the wind regime could dramatically alter flushing during the low flow season. Also, apart from the complex effects that are anticipated over estuarine biodiversity and ecosystem metabolism, increasing temperatures will lead to increased thermal stratification and to the expansion of the stratified season. This is rather critical because a prolonged isolation of the deep layer together with a reduction in the vertical exchange could result in anoxic events within the estuary. Likewise, reduced mixing would favor a concentration of nutrients in the upper layer and an enhanced biological activity. Finally, changes in precipitation timing and intensity can also alter the biological cycling. Rainfall in summer, when plankton communities are more responsive to external inputs, could result in intense blooms. Conversely, sustained drought in winter would suppose a loss of the estuarine circulation at Mahon. Decreased salinity areas represent an essential habitat for the reproductive ecology of several organisms in the Mediterranean Sea (Palomera 1992; Alemany et al. 2006).

In conclusion, Mahon is an anthropic system in which, as many other Mediterranean areas, phytoplankton seasonality has shifted from the typical winter bloom into a summer biomass maximum. Approximately 59% of the chlorophyll variability corresponds to no seasonal signal driven by event scale physical and biogeochemical processes such as flushing time variations or nutrient enrichment. Even so, the ecosystem seems to be quite resilient to these episodes. However, the summer season is a vulnerable period both because renewal depends critically on wind forcing, and because eventual rain episodes produce stronger biotic response than in other seasons. Alteration of the magnitude, frequency, and duration of these disturbance regimes in this season could provoke marked changes in the behavior of the estuary. These findings point to a critical issue that merits further investigation.

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Capítulo 2

Variación estacional y fuentes de metales traza disueltos en el puerto de Maó, isla de Menorca. / Seasonal variation and sources of dissolved trace metals in Maó Harbour, Minorca Island.

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Abstract

The environmental conditions of semi-enclosed coastal water-bodies are directly related to the catchment, human activities, and oceanographic setting in which they are located. As a result of low tidal forcing, and generally weak currents, waters in Mediterranean harbours are poorly renewed, leading to quality deterioration. Here, we characterise the seasonal variation of trace metals (i.e. Co, Cd, Cu, Fe, Mo, Ni, Pb, and Zn) in surface waters, and trace metal content in sediments from Maó Harbour, a semi-enclosed coastal ecosystem in the NW Mediterranean Sea. Our results show that most of the dissolved trace metals in the waters of Maó Harbour exhibit a marked inner - outer concentration gradient, suggesting a permanent input into the inner part of the harbour. In general, metal concentrations in the waters of Maó Harbour are higher than those in offshore waters. Concentration of Cu (21 ± 8 nM), Fe (9.2 ± 3.2 nM) and Pb (1.3 ± 0.4 nM) are particularly high when compared with other coastal areas of the Mediterranean Sea. The concentration of some metals such as Cu and Zn increases during summertime, when the human population and boat traffic increase during the tourism season, and when resuspension from the metal enriched sediments is higher. The evaluation of the metal sources in the harbour reveals that, compared with other putative sources such as runoff, aerosol deposition and fresh groundwater discharges, contaminated sediments are the main source of the metals found in the water column, most likely through vessel-driven resuspension events. This study contributes to the understanding of the processes that control the occurrence and distribution of trace metals in Maó Harbour, thus aiding in the effective management of the harbour, and enhancing the overall quality of the seawater ecosystem.

Highlights

- Distribution and trace metals fluxes in Maó Harbour are analyzed.
- Sediment resuspension is recognized as the main source of metals.
- Maó is identified as constant supplier of trace metal into coastal waters.
- The study will help in the effective ecological management of Maó.

2.1. Introduction

Coastal regions are highly variable and complex systems, with dynamics resulting from land-ocean interactions. These areas play an important role in the biogeochemical cycling of nutrients and metals, and serve as buffer zones between terrestrial and marine systems (Jahnke et al., 2008; Falco et al., 2010). Bays, harbours and other semi-enclosed areas have reduced water renewal while integrating the loads from large watersheds. These ecosystems show high sensitivity to environmental disturbances, both of natural and anthropogenic origin (e.g. Lucas et al., 1999; Cloern, 1999, Cullen et al., 2002).

Coastal marine waters are usually enriched in nutrients and trace metals compared with the open ocean (e.g., Kremling and Hydes, 1988; Kremling and Pohl, 1989; De Jonge et al., 1994; Nixon, 1995; Paerl, 1997; Pelley, 1998; Le Gal et al., 1999). This enrichment results from the direct influence of rivers, submarine groundwater discharge (SGD), atmospheric dust deposition, natural weathering, or anthropogenic sources discharging along the coast (e.g. Martin and Whitfield, 1983; Nixon 1995; Paerl 1997; Cotté-Krief et al., 2000; Tovar-Sanchez et al., 2014). The release of heavy metals from anthropogenic activities is usually the major cause of the increase in concentrations that may result in alterations to their natural geochemical cycles.

Although trace metals in the sea normally occur at low concentrations, they may have a considerable biological effect on the coastal biota (Rainbow, 1992). Some trace metals, such as Fe, Co or Mo, may influence the productivity and species composition of phytoplankton, while others may have a toxic effect (e.g. Pb, Cu; Sunda, 1989, Jordi et al., 2012; Echeveste et al., 2012). Moreover, above a certain threshold all bioavailable trace metals are potentially toxic (Kennish, 1996).

Aerosol deposition, riverine runoff and/or SGD have been identified as the major contributors to the dissolved pool of trace metals in the waters of the Mediterranean Sea (Guerzoni et al., 1999; Ludwig et al., 2009; Krom et al., 2004; Tovar-Sanchez et al., 2014; Rodellas et al., 2014; Trezzi et al. 2016). The study of metal fluxes from sediments (e.g. through diffusion, bioturbation, bioirrigation, or resuspension events) in nearshore areas have received scarce attention, even though they may represent an important source of metals with a significant effect on the global dynamics and functioning of phytoplankton (e.g. Lafabrie et al., 2013). This influence may be exacerbated in some coastal environments such as harbours and bays, where industrial activities have concentrated since the Industrial Revolution, dumping large amounts of contaminants that accumulated in sediments (Garcia-Orellana, et al., 2011; Gargouri et al., 2011; Lafabrie et al., 2007). Although industrial effluents are nowadays more tightly controlled, the accumulated contaminants in the seabed can still represent an important source of pollution, exceeding other sources.

Despite the importance of natural or anthropogenic disturbances, and physicochemical forcing in the regulation of ecosystem biogeochemistry, there is a lack of information about these processes which affect the dynamics of trace metals in low-energy confined coastal systems, such as harbours. The identification of trace metal sources in coastal ecosystems, and the processes controlling their seasonality are critical in the understanding of the ecological functioning and the biogeochemical cycles in the coast as well as for the application of government regulations on seawater quality. These studies are especially critical in the nearshore waters of oligotrophic areas, as is the case of the Mediterranean Sea, where a large proportion of the marine productivity is regulated by the supply of terrestrial input (Tovar-Sanchez et al., 2014).

The natural semi-enclosed harbour of Maó (Minorca Island, NW Mediterranean Sea) is a highly impacted region particularly vulnerable to variations in climate conditions and where, as a consequence of the industrial activities established along the harbour during the last century, metal concentrations have increased in the sediments (Garcia-Orellana et al., 2011). This study aims to identify processes and quantify fluxes that control the spatial and temporal distribution of trace metal composition (i.e. Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn) in waters and sediments of Maó Harbour. This research will contribute to the knowledge of the biogeochemical cycle of trace metals in the area which will help in the appropriate management of the port (e.g. dredging, maritime traffic) and will also enhance its ecological sustainability.

2.2. Material and methods

2.2.1. Site description

Maó Harbour (Minorca Island, Spain; 39°52′N 4°18′E; Fig. 2.1) is considered as one of the largest natural harbours in the Mediterranean Sea, with a length of ~5.5 km, a width of ~0.6 km and a depth of up to 30 m. The geomorphology of the area is conditioned by a NW-SE running geological fault over which the harbour is situated. Its mouth is very narrow (~300 m) and relatively shallow (~14 m), which greatly restricts the exchange with offshore waters. The climate in the area is characterised by mild winters (~13°C) and relatively hot and dry summers (~28°C). Annual precipitation is 563 ± 132 mm (Data from the Spanish Meteorological Agency, AEMET). The harbour receives terrestrial water input from different sources. Runoff from a watershed extending to the north is channelled through a small stream intermittently discharging into the harbour head. The input may be torrential during autumn and early winter when precipitation is at its highest. Likewise, submarine groundwater discharge, which includes fresh groundwater discharge through the limestone lithology extending along the southern coast of the harbour and seawater recirculating through sediments, may represent a source of trace metals (total flow $60 \cdot 10^3$ - $180 \cdot 10^3$ $m^3 d^{-1}$; Rodellas et al., 2015a).

Various types of industries (mainly marine, motor, textile, jewellery and footwear factories) have been established around the harbour during the two last centuries and have led to the dumping of waste-water into the bay, producing a progressive accumulation of metals in sediments (e.g. Ag, Cd, Cu, Ni, Pb; Garcia-Orellana et al., 2011). Since 1978, urban and industrial wastes are dumped into the open sea through an outfall pipe. Nowadays, Maó Harbour is an important tourism destination and hosts the most important industrial, maricultural (mussels) and mercantile centre of the island. As a consequence, cruise boats and deep draft vessels daily transit the waters of the harbour, with higher frequency during the summer.

2.2.2. Sampling and analysis

Surface water samples were collected during four surveys in July 2010, October 2010, March 2011 and June 2011. In all surveys 15 stations were sampled in a longitudinal transect following the main axis of the harbour (Fig. 2.1). At each station a SBE-25 CTD was deployed from surface to the bottom in order to characterise the hydrographic properties of the water column. We use practical salinity scale to report salinities and therefore salinities values are expressed by dimensionless number (PSS-78, UNESCO 1981).

Water samples were collected at 1 m below the surface using a peristaltic pumping system equipped with acid-washed C-Flex tubing in the pump head, and filtered in-situ through an acid-cleaned polypropylene cartridge filter (0.22 µm; MSI, Calyx®). The sampling protocol has been described elsewhere (Tovar-Sánchez, 2012). Additionally, samples for trace metals analysis were also collected from the stream inflowing into the inner harbour (in October 2010 and March 2011) and from the waters of eight nearshore wells (W1-8) (Fig. 2.1). Waters from these wells are assumed to be representative of the groundwater inflow into the harbour (Rodellas et al., 2015a).

Dissolved trace metals samples were acidified to pH <2 with ultrapure grade HCl (Merck) in a class-100 HEPA laminar flow hood and stored for at least 1 month before extraction. Concentrations of metals (i.e. Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn) were analysed with ICP-MS (PerkinElmer ELAN DRC-e) after a pre-concentration using the APDC/DDDC organic extraction method (Bruland et al. 1979; Tovar-Sánchez, 2012). The accuracy of the analysis was established using Coastal Seawater Reference Material for trace metals (NASS-5, NRC-CNRC) (obtained recoveries of 108%, 93%, 107%, 93%, 97%, 92%, 88%, and 105% for Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn, respectively). The limits of detection, calculated as three times the standard deviations of subsequent blank measurements, were 6, 4, 141, 129, 309, 22, 4, 427, and 287 pM for Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn, respectively.

Surface sediment samples (1-2 cm) were collected by scuba divers in June 2011, in 6 stations located along the harbour (Fig. 3.1). Samples were stored frozen in polyethylene tubes until analysis. The grain size in each sample was determined with a set of stainless steel sieves for six fractions with an electric sieve. Samples were treated initially with hydrogen peroxide to remove the organic fraction (McManus, 1988; Walling and Woodward, 1993) and dried in an oven at 60°C for two days. Approximately 50 g of sample were sieved for a period of 15 min using a battery of 6 sieves with mesh sizes of: 2 mm, 1 mm, 500 µm, 250 µm, 125 µm, and 63 µm. Material retained on each sieve was weighed to calculate the percentage in each size fraction.

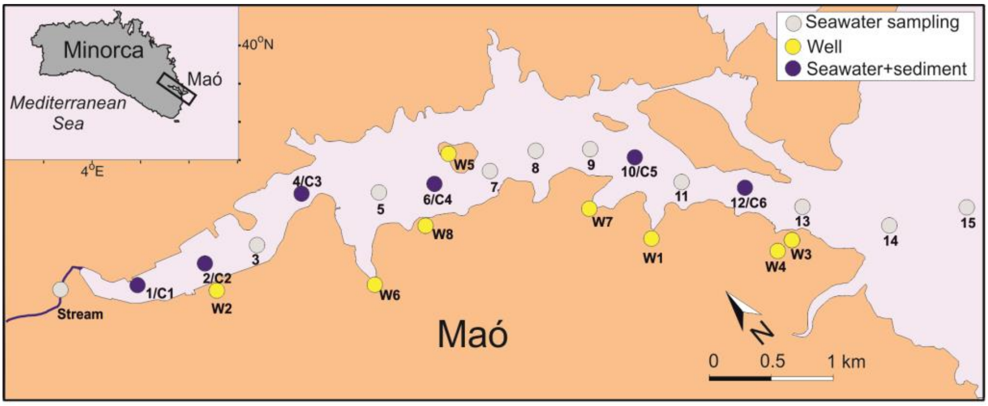

Figure 2.1 Map showing the location of the study area in Minorca Island (North-Western Mediterranean Sea), and the sampling stations of surface water (1 to 15), wells (W1 to W8) and sediments (C1 to C6).

Metal concentrations in surface sediments (i.e. Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn) were determined by ICP-OES (Perkin Elmer ICP-OES Optima 5300 DV). Samples were dried in an oven at 60°C for two days and subsequently ground in a zirconia ball mill (10 min at 170 rpm) before analysis. Metals were extracted with a microwave acid digestion system (CEM model Mars 5) according to the SW-846 EPA Method 3051A (US EPA 1987), which involved the digestion of 0.2 g of sediment sample by triplicate with 10 mL of nitric acid (65%, Suprapur quality) in Teflon vessels. After digestion, samples were diluted to 50 mL using Milli-Q water and then analysed. The accuracy of the analysis was checked with the certified reference material PACS-2 (National Research Council Canada), with recoveries of 99.4%, 99.9%, 90.9%, 86.7%, 92.6%, 89.8%, 91.6%, and 93.3% for Cd, Co, Cu, Fe, Mo, Ni, Pb, and Zn respectively.

2.2.3. Statistical analysis

Statistical evaluation was performed using SigmaPlot 12.0 for Windows. Previous to the selection of the statistic test a Shapiro-Wilk test was previously performed for each case to evaluate the normality of the data distribution. The non-parametric Kruskal-Wallis One Way Analysis of Variance on Ranks was carried out to determine differences in average metal concentrations between the four surveys. Because the small number of data for each survey and their normal distributions, a Student's t-test was employed to identify differences in metal concentrations between inner and outer harbour. Correlations between ^{224}Ra and trace metals through the four surveys were tested using a linear regression model that also involved the calculation of Pearson Product Moment Correlation Coefficient. A confidence interval of 95% was adopted and results for all tests were considered significant if associated p values were <0.05.

2.3. Results and discussion

2.3.1. Hydrologic characteristics

Marked seasonal differences in seawater temperature and salinity were observed between surveys (Fig. 2.2). During summer (July 2010 and June 2011), and in the deepest parts of the harbour, the water column presented a marked thermal stratification (Fig. 2.2A and G), varying from 25 °C in the surface to <17 °C near the bottom. The thermocline was located at 14.0 ± 1.6 m and presented a strong temperature gradient. Lower temperatures were measured during October 2010 (~ 20 °C) and March 2011 (~14 °C) and when the water column was more homogeneously mixed with a small temperature variation of < 2 °C between the sea surface and the bottom (Fig. 2.2C and E). Salinity in the harbour waters ranged between 38.1 in July 2010 and 37.4 in March 2011 when a reduced salinity layer was observed in the upper 5 m of the water column, which was indicative of enhanced runoff. Differences in salinity between the harbour and offshore waters were also observed in July 2010 and October 2010 (t-test: t value 6.62 and -7.72 for July 2010 and October 2010, respectively. p value: < 0.001, degrees of freedom (DF): 13, n 'inner': 7, n 'outer': 8). The inner part experienced increased salinities in July, possibly as a consequence of intense evaporation (Fig. 2.2B). Conversely, precipitation and runoff decreased the salinity during October (Fig. 2.2D). These salinity differences suggest water confinement in the harbour and, consequently, the environmental conditions appear to be driven by local processes rather than by general oceanographic conditions.

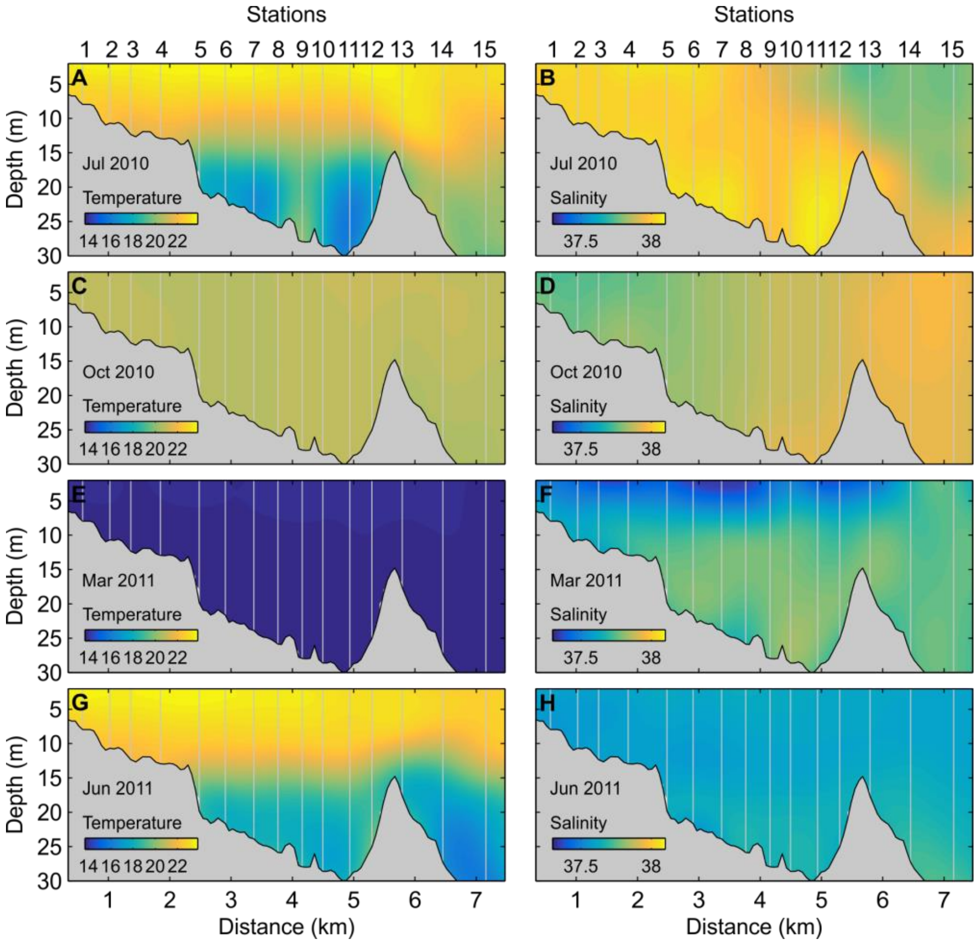

Figure 2.2 Temperature (°C) and salinity along the Maó Harbour transect for the four conducted surveys. The x-axis indicates the distance from the inner shore of the port.

2.3.2. Trace metals in Maó Harbour

2.3.2.1. Metals in surface seawater

With the exception of Cd and Mo, all dissolved metals exhibited an inshore-offshore negative gradient with significantly (t-test: t values range from 7.51 for Cu to 2.48 for Fe. p value: <0.001, DF = 13, n 'inner': 7, n 'outer': 8) higher concentration of Co (0.39 ± 0.05 nM), Cu (20 ± 4 nM), Fe (12 ± 5 nM), Ni (4.8 ± 0.3 nM), Pb (1.6 ± 0.2 nM) and Zn (34 ± 6 nM) in the inner basin of the harbour (stations 1 to 7), relative to those concentrations measured at the outer basin (stations 8 to 15; Co: 0.22 ± 0.04 nM, Cu: 13.1 ± 4.3 nM, Fe: 7.2 ± 1.6 nM, Ni: 3.8 ± 0.3 nM, Pb: 1.0 ± 0.2 nM, and Zn: 14 ± 5 nM) (Figs. 2.3 and 2.4). The enhanced concentrations in the inner harbour suggest that the main sources of trace metals were likely located at this site. Cobalt, Cu, Ni and Zn correlated well in the four surveys (coefficient of determination $r^2 > 0.83$), suggesting a common origin. Copper and Zn proportionally increased during the summer (July 2010 and June 2011) when tourist population increases, and when navigation in the harbour is at its highest. In general, lower variations were measured during March 2011, which is attributed to an increased flushing of surface waters during winter rainfall episodes.

While in most of the cases metal concentrations decreased linearly offshore, in some other cases marked variations occurred between St 9 and St10. This was noticeable during March 2011 when the concentrations of Co, Ni, Pb and Zn increased at this location and, more remarkably, in the case of Pb in October 2010. These variations suggest a point source in this area either related to uncontrolled urban discharge or to enhanced groundwater flow. This last source is less likely since the well W7, located in this area, did not show particularly high Pb concentrations (i.e. 2.3 - 2.7 nM).

Unlike other metals, concentrations of Mo and Cd showed a rather constant distribution along the harbour, but with a remarkable seasonal variation (Kuskal Wallis test: H values 48.72 for Cd and 43.43 for Mo, p value: <0.001, DF: 3, n: 15). The highest concentrations were found in March 2011 (Cd: 0.39 ± 0.01 nM and Mo: 137 ± 3 nM) and the lowest in July 2010 for Cd (0.17 ± 0.02 nM) and July 2011 for Mo (108 ± 5 nM) (Figs. 2.3A and 2.4A). The distribution and behaviour of Cd and Mo are consistent with those found in other studies showing that in estuarine and coastal areas, the input from rivers and terrestrial sources is not the origin of these elements (Tovar-Sanchez and Sañudo-Wilhelmy, 2011). Seasonal variations may be controlled by other chemical and environmental conditions such as solubility, complexation by organic ligands, atmospheric inputs, etc. (Migon et al., 2002; Heimbürger et al., 2014).

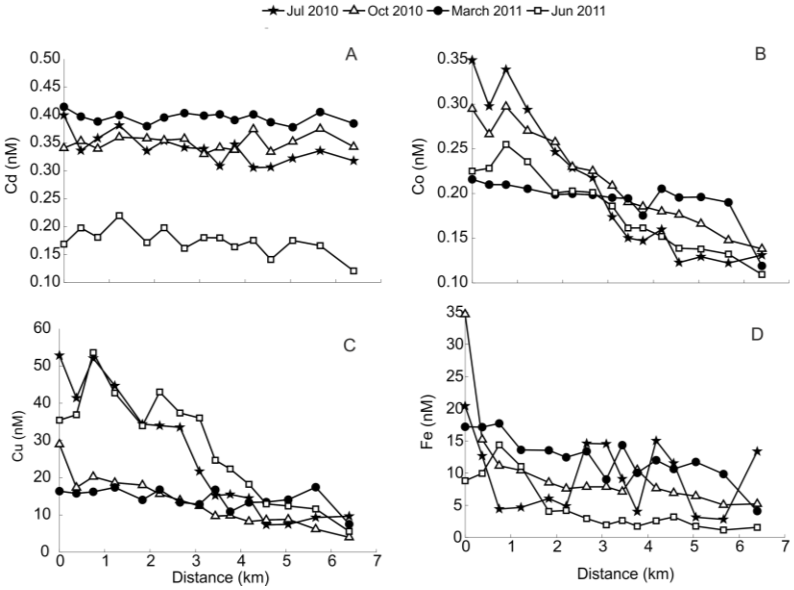

Figure 2.3 Trace metal concentrations along the Maó Harbour for all four surveys. (A) Cd; (B) Co; (C) Cu and (D) Fe. The x-axis indicates the distance from the inner shore of the port.

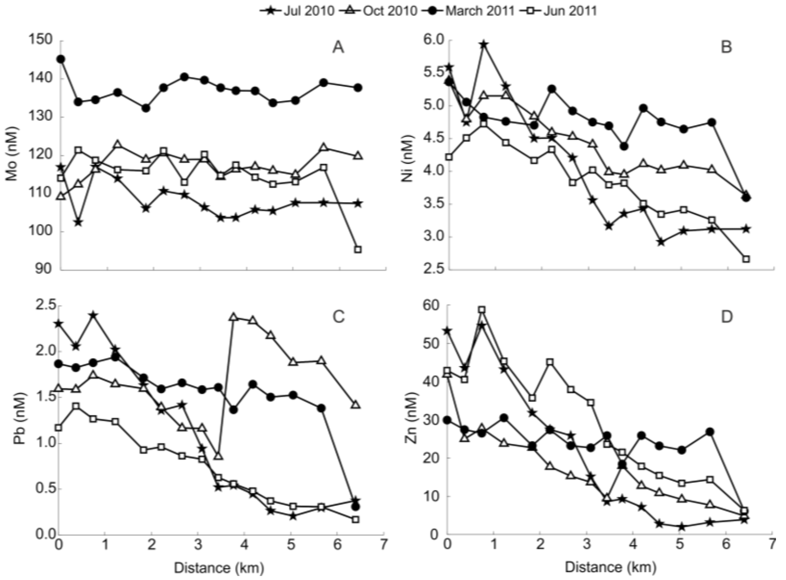

Figure 2.4 Trace metal concentrations along the Maó Harbour for all four surveys. (A) Mo; (B) Ni; (C) Pb and (D) Zn. The x-axis indicates the distance from the inner shore of the port.

The concentrations of dissolved metal in the waters of Maó Harbour are higher than those reported for different areas of the Mediterranean Sea and Spanish coasts with the highest differences measured for Cu (21 ± 8 nM), Fe (9 ± 3 nM) and Pb (1.3 ± 0.4 nM) (Table 2.1). The progressive sediment contamination by metals (i.e. Pb and Cu) due to the various types of industry established around the harbour (Garcia-Orellana et al. 2011) could be the cause of these high concentrations measured in its waters.

Table 2.1 Range of dissolved metal concentrations in seawater from different areas of the Mediterranean Sea and Spanish coast. Numbers in parenthesis are average ± standard deviation. (ver anexos p. 188)

	Cd (nM)	Co (nM)	Cu (nM)	Fe (nM)	Mo (nM)	Ni (nM)	Pb (nM)	Zn (nM)
Ferrol Ria [a]	0.09 - 0.10		6.7 - 9.2				0.20 - 0.30	17 - 24
Vigo Ria [b]	0.02 - 0.10 (0.05 ± 0.02)	0.30 - 1.6 (0.67 ± 0.37)	1.1 - 5.3 (5.6 ± 2.4)			1.7 - 7.8 (4.1 ± 1.5)	0.08 - 0.25 (0.18± 0.07)	7.4 - 19 (13 ± 4)
Palma beach [c]			5.9 - 13	3.0 - 6.0	81 - 160	2.9 - 5.6	0.28 - 0.40	3.3 - 10
Cadiz bay [d]	(0.10 ± 0.03)		(6.7 ± 3.9)				(230 ± 150)	(17 ± 9)
Algeciras bay [d]	(0.2 ± 0.1)		(17 ± 8)				(14 ± 2)	(130 ± 60)
Majorca bays [e]	0.30 - 0.37	0.16 - 0.29	3.9 - 11	5.5 - 12	117 - 130	3.6 - 4.6	0.07 - 0.19	3.1 - 8.0
Majorca Shelf water [f]	0.34 - 0.37	0.14 - 0.17	4.5 - 9.3	2.4 - 3.6	120 - 133	3.7 - 4.2	0.10 - 0.13	2.0 - 6.6
Western Mediterranean [f]	0.06 - 0.08	0.05 - 0.17	1.1 - 2.3	1.6 - 8.0	107 - 140			
Eastern Mediterranean [f]	0.07 - 0.09	0.08 - 0.14	1.4 - 1.9	1.9 - 6.1	115 - 1230			
Aegean Sea [f]	0.08 - 0.12	0.12 - 0.29	1.8 - 4.4	2.1 - 4.0	110 - 130			
Marmara Sea [f]	0.10 - 0.11	0.45 - 0.52	7.7 - 8.6	3.7 - 4.9	55 - 61			
Black Sea [f]	0.06 - 0.09	0.18 - 0.37	6 - 8.8	1.8 - 3.7	39 - 47			
Maó Harbor [g]	0.1 - 0.4 (0.3 ± 0.1)	0.1 - 0.6 (0.30 ± 0.03)	2 -54 (21 ± 8)	1.2 - 35 (9.2 ± 3.2)	95 - 150 (119 ± 12)	2.7 - 5.9 (4.3 ± 0.4)	0.2 - 2.4 (1.3 ± 0.4)	2 - 59 (23 ± 5)

[a]Cobelo-García et al. (2005); [b]Santos-Echeandías et al. (2009); [c]Rodellas et al. (2014); [d,]Morillo et al. (2015); [e]Tovar-Sanchez et al. (2014a); [f]Tovar-Sanchez et al. (2014b); [g]Present study

2.3.2.2. Metals in surface sediments

Trace metals concentrations in surface sediments of Maó Harbour varied between 1 - 6 $\mu g\ g^{-1}$ (Co), 2 - 81 $\mu g\ g^{-1}$ (Cu), 0.2 – 2.3% (Fe), 1 – 22 $\mu g\ g^{-1}$ (Ni), 14 – 124 $\mu g\ g^{-1}$ (Pb), 17 – 134 $\mu g\ g^{-1}$ (Zn), (Table 2.2). The dominant grain size in most samples was silty-sands although, in the inner basin, in proximity to the urban area (C2 and C3 at Fig. 2.1), the proportion of silts exceeded 50% (sandy-silts). No direct relationship was found between metal content in sediments and grain size. Only concentrations measured at C4 site were significantly different (t-test: t values ranged from 19.13 for Co to 70.0 for Cu, p value: < 0.001, DF: 4, n: 5) from the rest of the samples, exhibiting lower metal contents. This station was also characterised by the highest content of shells and other carbonated structures (Ca: 8 – 26%) with lower metal binding capacity. Cobalt, Fe and Ni concentrations in the surface sediments of Maó Harbour were similar to those reported for other Mediterranean bays (Table 2.3). Sediments were particularly enriched in Cu, Pb and Zn with measured concentrations of up to 28 – 75%, 42 – 95% and 12 – 48%, higher than in other Mediterranean areas (Table 2.3).

Table 2.2 Average metal concentrations (Av) ± standard deviation (SD) (n = 3) and grain-size in surficial sediments from Maó Harbour. Metal concentrations are expressed in μg g-1, except Fe that is in % (dry weight). (ver anexos p. 189)

Stations	Cd		Co		Cu		Fe		Mo		Ni		Pb		Zn		Grain size		
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	<63 mm (%)	>63 mm(%)	
C1	nd	-	3,3	0,5	75,2	1,2	1	0	1,7	0,5	9,7	0,6	42,4	1,1	93,5	0,4	20	80	silty-sand
C2	nd	-	5,7	0,2	40,8	0,7	1,9	0	2,3	1,1	16,4	0,2	124	3	77,2	1,1	57	43	sandy-silt
C3	0,1	0,1	3,0	0,2	33	2	0,90	0	1,0	1,0	7,6	0,4	57	2	67	4	53	48	sandy-silt
C4	nd	-	0,85	0,14	2,1	0,2	0,2	0	1,1	0,6	1,4	0,6	14	2	17,1	0,8	23	77	silty-sand
C5	nd	-	4,5	0,4	48,0	0,6	1,6	0,1	2,6	1,0	16,2	0,3	93	6	91	2	35	65	silty-sand
C6	0,1	0,1	5,64	0,04	72	2	2,1	0,1	1,6	0,9	19,8	0,7	113	3	123	3	33	67	silty-sand

Av: average; SD: standard deviation

Table 2.3 Mean values ± standard deviation (SD) of metal concentrations in surficial sediments of different areas of Mediterranean Sea. Concentrations are expressed in μg g-1 (dry weight), except Fe (%). (ver anexos p. 190)

Element	Gulf of Naples (Italy)[a]	Sfax Coast (Tunisia)[b]	Sardinia. Italy[c]	Corsica. France[c]	Toscana. Italy[c]	Ionian Sea. Southern Italy[d]	Maó Harbour[e]
Cd	0.57 ± 0.62	5.9 ± 0.5	0.07 ± 0.03	0.03 ± 0.00	0.40 ± 0.10	-	0.09 ± 0.00
Co	-	-	2.5 ± 0.0	55 ± 14	7 ± 1	-	5 ± 1
Cu	27 ± 29	16 ± 4	-	-	-	47 ± 3	65 ± 18
Fe	15 ± 13	4.9 ± 0.4	-	-	-	3.2 ± 0.3	1.9 ± 0.5
Mo	-	-	-	-	-	-	1.7 ± 0.2
Ni	7 ± 10	14 ± 8	4 ± 1	1 ± 5	40 ± 6	52 ± 3	18 ± 5
Pb	220 ± 170	32 ± 17	19 ± 2	5 ± 1	45 ± 5	57 ± 8	98 ± 25
Zn	600 ± 550	59 ± 17	-	-	-	100 ± 13	110± 30

[a] Romano et al., 2004; [b]Gargouri et al., 2011; [c] Lafabrie et al., 2007; [d] Buccolieri et al., 2006; [e]Present study.

2.3.3. Sources of metals in Maó Harbour waters

Several trace metal sources can be identified in the harbour waters. Diversions from urban drainage systems, and groundwater discharges through the porous limestone substrate along the southern coast of the harbour could represent a source of metals for the harbour. A stream located in the innermost part of the harbour is the most important source of freshwater runoff, though the evacuation through the city storm-water drainage system could be also significant during the heaviest rainfall events. Median concentrations of metals in the stream were higher than those measured in the inner harbour waters, suggesting an influence of this stream on overall metal budgets (Co: 4.4 ± 3.2 nM, Cd: 0.2 ± 0.2, Cu: 53.9 ± 7.1 nM, Fe: 677 ± 375 nM, Mo: 37.5 ± 29.5 nM, Ni: 62.4 ± 2.1 nM, Pb: 1.9 ± 0.2 nM, and Zn: 70.9 ± 8.5 nM). Table 2.4 shows the estimated annual metal fluxes from the stream considering a drainage area of $2.8 \cdot 10^{7}$ m^{2}, an impervious area of $5.0 \cdot 10^{5}$ m^{2}, an average precipitation of 563 mm y^{-1} and a calculated flow of $3 \cdot 10^{6}$ m^{3} y^{-1}. Since differences of Mo and Cd with offshore waters were not significant they were not included in the different fluxes estimations.

Table 2.4 Average annual flows in mol y-1 of the different metal sources to the Maó Harbour. Numbers in parenthesis are the contribution in percentages of the total flow. (ver anexos p. 191)

	Co		**Cu**		**Fe**		**Ni**		**Pb**		**Zn**	
Groundwater	13 ± 11	(2)	160 ± 70	(0.3)	1400 ± 900	(6)	160 ± 130	(3.7)	17 ± 23	(0.6)	600 ± 940	(0.7)
Stream waters	13 ± 12	(2)	160 ± 80	(0.3)	2000 ± 1500	(9)	180 ± 90	(4.1)	6 ± 3	(0.2)	210 ± 110	(0.3)
Atmospheric deposition[*]	1	(0)	10	(0)	1200	(5)	4	(0.1)	4	(0.1)	550	(0.7)
Sediments	620 ± 15	(96)	59500 ± 100	(99.4)	18600 ± 1700	(80)	4000 ± 160	(92.0)	3000 ± 20	(99)	76000 ± 33000	(98)
Export offshore	650		60000		23200		4400		3000		78000	

[*] Maximun value from Guieu et al, 1997

Atmospheric deposition could be also an important source of trace metals in this region (e.g. Jordi et al., 2012). Direct atmospheric metal fluxes in Maó Harbour can be calculated from reported atmospheric fluxes (mol km^{-2} y^{-1}) in the NW Mediterranean Sea (Cd: 1.9 - 8.0, Co: 0.3 - 2.2, Cu: 13 - 20, Fe: 575 - 2525, Ni: 6.8 - 8.0, Pb: 4.6 - 9.0, and Zn: 11 - 1150; Guieu et al., 1997) considering the water body surface area (~ 2.3 km^{2}; Table 2.4). Additionally, it has been demonstrated that submarine groundwater discharge (SGD) may deliver a substantial amount of trace metals into the sea, and in some areas represents a major source of these compounds to coastal ecosystems (Windom et al. 2006; Beck et al. 2007; Rodellas et al. 2014; Tovar-Sánchez et al., 2014; Trezzi et al., 2016). Here, we differentiate between fresh groundwater input, which is the only SGD fraction supplying external input of water, and metals to the harbour, and seawater recirculation through sediments. In order to evaluate the influence of fresh groundwater discharge, we measured the concentrations of metals in 8 wells located around the harbour (Average ± SDV; Cd: 0.1 ± 0.3 nM, Co: 4.2 ± 3.5 nM, Cu: 53 ± 23 nM, Fe: 460 ± 280 nM, Mo: 15 ± 9 nM, Ni: 54 ± 41 nM, Pb: 6 ± 8 nM and Zn: 190 ± 310 nM; Fig S2.1). The flux of metals supplied by fresh groundwater discharge was calculated by multiplying the concentrations measured in the wells by the estimated annual terrestrial groundwater flow into Maó Harbour ($3.3 \cdot 10^{6}$ m^{3} y^{-1}) (depicted from island-scale hydrographic budget calculations (DGRH, 2013) and assuming a drainage area of 27.9 km^{2}. The resulting fresh groundwater flow represents some 7% of total SGD measured by Rodellas et al. (2015a) in Maó Harbour using Ra isotopes, suggesting that > 90% is recirculated seawater, which, is consistent with reports from other Mediterranean areas (Rodellas et al., 2015b). Thus, the seawater recirculation, through harbour sediments can also contribute to the transfer of metals from sediments into the water column, and thus it is included as benthic fluxes.

Input of metals from sediments can occur by diffusion, bioirrigation, desorption, resuspension of sediments, and seawater recirculation (also referred to as pore-water transfer) driven by pressure gradients mainly forced by seiches, tides, waves, bottom currents, benthic organisms, storms, or density-driven convection. Additionally, intense sediment resuspension occurs in the Harbour as the result of the navigation of deep-draft vessels (Fig. S2.2). This mainly occurs in the shallow areas of the inner basin where the vessels manoeuvre, and where the bottom sediments have been affected by progressive contamination (Garcia-Orellana et al., 2011).

In order to evaluate the importance of each metal source, as well as to try and understand the role of sediments in the input of metals in the water column, several approaches can be taken. Trace metal input from sediments into the water column is usually evaluated performing laboratory experiments (Kalnejais et al., 2010; Durán et al., 2012), measuring in-situ fluxes at the water-sediment interface (Viollier et al., 2003) or monitoring continuously concentrations of trace metals in the water column (Superville et al., 2014), among others. Here, metal fluxes from sediments into water column were evaluated through a mass balance of the studied compounds (i.e. comparing major sources and sinks of the metals analysed, where the only unknown is the input from sediments). In order to obtain a conservative estimate, the fluxes of metals from sediments into the waters were estimated by calculating the difference between the offshore export and the sum of fluxes of the major inputs (groundwater discharge, atmospheric deposition and stream inputs) (Table 2.4). It is noteworthy that we assume that major net output of trace metals from the harbour derive from the export to the open sea (offshore exports), and therefore output related to the rapid (relative to residence time) scavenge of dissolved trace metals by suspended particles, organic matter and Fe- and Mn- hydroxides, and biological consumption were excluded from the mass balance. Including the output would considerably increase the fluxes of trace metals from sediments. The offshore metal export can be estimated from the excess metal inventory in the harbour (i.e. the difference between the average concentrations in the harbour (St 1-14) and the outer station (i.e. St 15) times the harbour volume (i.e. $38.3 \cdot 10^6$ m^3), divided by the residence time of dissolved compounds in harbour waters (from 2 to 7 days, depending on the season Rodellas et al., 2015a) (Table 2.4). This qualitative and conservative comparison allowed us to determine that the input from sediments represent the main source in the Harbour, being 96% for Co, 99% for Cu, 80% for Fe, 92% for Ni, 99% for Pb and

98% for Zn of the total flux into the harbour waters, and revealing the importance of the supply of trace metals from contaminated bottom sediments.

Although several processes may be responsible for these metal fluxes from sediments (e.g. diffusion, bioturbation, bioirrigation, pressure-induced advection), sediment resuspension triggered by maritime traffic may represent the dominant mechanism, when the frequency and magnitude of the resuspension events are taken into account. Maó Harbour is an important mercantile centre and touristic destination where deep-draft vessels are daily steaming along the harbour, especially during the summer season. The shallowness of the inner part of the harbour (transit channel of 10 - 14 m depth) and the draft of the vessels (6 to 9 m) produce the resuspension of significant amounts of sediments (Fig. S2.2), which encourages the transfer of contaminants from the sediments into the water column (Garcia-Orellana et al. 2011; Rodellas et al., 2015a). This hypothesis has been recently tested by Rodellas et al. (2015a), identifying sediments in the Maó Harbour as a major source of short-lived Ra isotopes (^{223}Ra and ^{224}Ra) into the water column that are continuously produced in sediments by their parent disintegration (^{227}Th and ^{228}Th, respectively). Resuspension events triggered by vessel docking manoeuvres represented a significant contributor to the short-lived Ra budgets of the water column. Indeed, concentrations of these short-lived Ra isotopes in waters measured by Rodellas et al. (2015a), which were collected at the same stations and concurrently with metal samples, are well correlated with metal concentrations in harbour waters, except for Cd and Mo that have different sources, (coefficients of determination, r^2, ranging from 0.98 for Fe to 0.999 for Co, $p < 0.001$) (Fig. 2.5). These correlations suggest that short-lived Ra isotopes and metals are supplied by the same source, pointing to the relevance of sediments as a source of the trace metals found in the water column, most likely through the resuspension events.

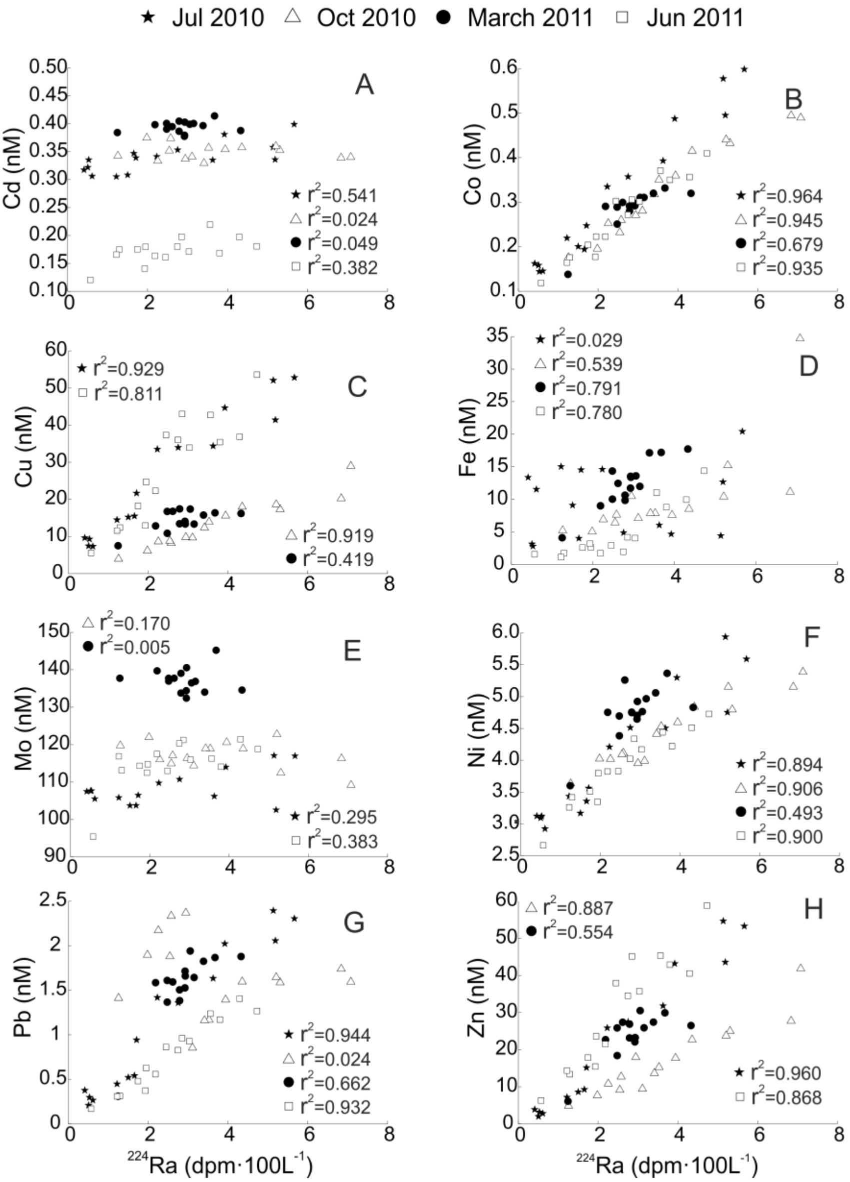

Figure 2.5 Scatter Plot of the concentrations between 224Ra and trace metals. (A) Cd; Co (B); Cu; (C) Fe; (D); Mo; (E); Ni (F); Pb (G) and (H) Zn. r2 values are the squared Pearson correlation coefficient.

2.4. Conclusions

The results presented here show that Maó Harbour, a semi-enclosed ecosystem with restricted exchange, exhibit a general onshore-offshore negative gradient of dissolved metals parts during the four seasons. Contaminated sediments represent the main source of the Co, Cu, Fe, Ni, Pb and Zn found in the water column, most likely as the result of their resuspension driven by maritime traffic. As a consequence of metal contamination in the sediment, and the continuous transfer with the water column, the levels of Cu and Pb in Maó Harbour are considerably higher than the values reported for the sediment and waters of other Mediterranean areas. The effect of the input of trace metals from these anthropogenic sources on the ecological functioning of the Maó Harbour deserves further analysis.

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Capítulo 3

Variación específica de la especie en las fuentes nutricionales de fósforo del microfitoplancton en un estuario del Mediterráneo / Species-specific variation in the phosphorus nutritional sources by microphytoplankton in a Mediterranean estuary

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Abstract

We investigated the species-specific phosphorus (P) nutritional strategies in the microphytoplankton community in the Mahon estuary (Minorca, Western Mediterranean) in 2011, under two contrasting hydrographic scenarios. Estuarine flow, nutrient concentrations, phytoplankton community composition and enzyme-labeled fluorescence (ELF) were measured in June and October, corresponding to the beginning and the end of summer. Dissolved inorganic nitrogen (DIN) and inorganic phosphate (Pi) exhibited enhanced concentrations in the inner estuary where N:P molar ratios suggested P-limitation in both surveys. Pi was low and variable (0.09±0.02 µmol L-1 in June and 0.06±0.02 µmol L-1 in October), whereas organic phosphorus remained a more readily available P source. Even though ambient Pi concentrations were slightly higher on June, when the microphytoplankton assemblage was dominated by dinoflagellates, the percentage of ELF labeled cells was notably higher (65±9% of total cells) than in October (12±10%), when the presence of diatoms characterized the microphytoplankton community. Alkaline phosphatase activity (AP) was mainly expressed by dinoflagellate taxa, whereas diatoms only displayed significant AP in the inner estuary during the June survey. A P-addition bioassay in which response of AP to Pi enrichment was evaluated showed marked reduction in AP with increasing Pi. However, some dinoflagellate species maintained AP even when Pi was supplied in excess. We suggest that in the case of some dinoflagellate species AP is not as tightly controlled by ambient Pi as previously believed. AP activity in these species could indicate selective use of organic phosphorus, or slow metabolic response to changes in P forms, rather than physiological stress to low Pi availability. We emphasize the importance of identifying the species-specific phosphorus requirements and their capability for P assimilation in order to understand the contribution of microphytoplankton to the whole community response to biogeochemical perturbations.

3.1. Introduction

The escalating input of nutrients from anthropogenic activities near the coast is considered as one of the greatest threats to worldwide coastal ecosystems. The consequences of this process include changes in species composition, reduction in aquatic biodiversity, alteration of food webs, uneven growth of primary producers, including algal blooms, with an increase of respiration rates in response to the rapid production of organic matter (Nixon, 1995; Cloern, 2001). These effects are pervasive in coastal aquatic systems, and reveal a major influence of nutrient availability on the distribution of marine microbial communities. Predicting and managing the effects of eutrophication requires an understanding of these nutrient-algae relationships. However, the complexity posed by the influence of various physical and bio-chemical factors makes the implementation of effective nutrient management strategies a challenging prospect (e.g. Carstensen et al., 2011).

Bottom-up control of phytoplankton growth is exerted through the imbalance between supply and demand of each nutrient involved in cell physiology (Conley et al., 2009). In spite of the fact that the co-limitation between N and P is not rare in a number of world areas, several studies emphasize the importance of phosphorus (P) as the principal limiting nutrient in the open ocean regions, and in some nearshore and estuarine environments (e.g. Krom et al., 1991; Thingstad et al., 1998; Ammerman et al., 2003). Traditionally, the inorganic P (P_i) form orthophosphate was considered as the sole form of P that could be utilized by phytoplankton to satisfy cellular requirements (Cembella et al., 1984). However, it is now widely accepted that despite the preferential use of orthophosphate, many phytoplankton taxa have the capability to utilize dissolved organic phosphorus (DOP) and other Pi forms such as polyphosphate (Currie and Kalff, 1984; Cotner and Wetzel, 1992; Nicholson et al., 2006).

In order for phytoplankton cells to take up DOP they must first hydrolyze the labile fraction into orthophosphate (Hoppe, 1993; Jansson et al., 1988). This process is mediated mainly by alkaline phosphatase (AP), an enzyme that has broad substrate specificity and hydrolyzes ester bonds between P and organic molecules (Cembella et al., 1984; Huang et al., 2005). In many phytoplankton species AP is regulated (induced or repressed) by ambient P_i concentrations, or by intracellular concentrations (Chrost and Overbeck, 1987; Nicholson et al., 2006). AP activity is therefore considered as indicative of P_i limitation. Indeed, AP activity has been used to indicate phytoplankton P-stress in both freshwater and marine environments (Cotner et al., 2000; Hernandez et al., 2002; Vidal et al., 2003).

Previous research on marine AP activity used a bulk enzyme assay method focusing on areas with low Pi concentrations or elevated nitrogen (N) to P ratios (Li et al., 1998; Cotner et al., 2000; Ivancić et al., 2010). In addition, the technique of single cell enzyme-labeled fluorescence (ELF) was developed to determine which specific cells within a phytoplankton community are using AP (Dyhrman and Palenik, 1999; Nicholson et al., 2006). The phytoplankton cells existing in a particular environment may respond in different ways to the P availability in the medium, depending on their previous physiological state and their capacity to take up the nutrients rapidly, thereby displaying diverse AP responses.

The objective of this study is to examine the species-specific assimilating capacity of the available P forms (inorganic/organic) by the microphytoplankton cells at Mahon estuary (Balearic Islands, Mediterranean Sea), and its relationship to the hydrodynamic conditions. In the Balearic Islands, available nitrogen concentrations are frequently higher than those of other elements, as result of human activities, and the low concentrations of P_i limit coastal productivity (Basterretxea et al., 2007, 2010; Tovar-Slating capacity of the available P forms (inorganic/organic) by the microphytoplankton cells at Mafor understanding phytoplankton growth and community composition along the Mediterranean coasts. Furthermore, the ability of some microphytoplankton species to switch between Pi and DOP sources may explain the sustainability of primary production during summer stratified conditions and, through trophic interactions, the subsistence of other components of the coastal food web (such as zooplankton and fish larvae).

3.2. Data and methods

3.2.1. Site Description

Mahon is an elongated (5.5 km long) and deep (>30 m) estuary on the east coast of Minorca (Balearic Islands, Mediterranean Sea; Figure 3.1). The geomorphology of the estuary is conditioned by a NW-SE running geological fault over which the estuary settles. The inner part of the estuary is shallow with mean depths of < 12 m, and contains a commercial harbor. Drains from the municipal network of Mahon concentrate in this area. The center part of the estuary is deeper (> 15 m) around a narrow band to the south of Isla del Rey, and shallower to the north. In this section of the harbor population concentrates in the town of Es Castell. The mouth of the harbor is very narrow (~300m) and a sill greatly restricts the exchange with offshore waters.

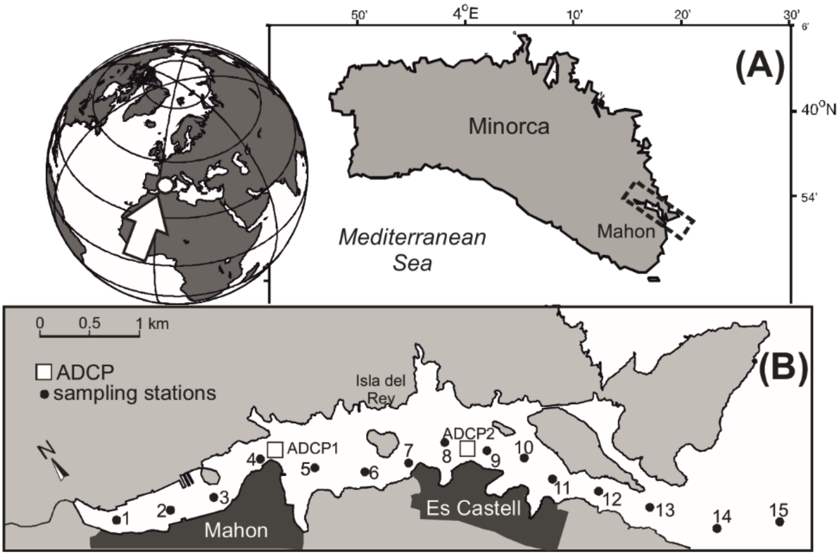

Figure 3.1 (A) Map showing the location of Mahon in Minorca Island (Mediterranean Sea) and (B) the sampling stations (black dots) and location of the ADCPs (open squares).

Climate in the area is characterized by mild winters and relatively hot and dry summers. Mahon receives fresh water inputs through groundwater seepage, and irregular surface flows in the head of the estuary that may be torrential during fall and early winter. Conversely, low freshwater inflow, together with strong evaporation during summer, may result in water circulation reversals when estuarine salinity exceeds that of the ocean. These low-inflow reversing estuaries are common in many temperate and warm world areas, including the Red Sea, California, South Africa, Western Australia and the Mediterranean Sea (Largier et al., 1996, 1997). In addition, water renewal in the Mahon estuary is generally poor because it is sheltered from coastal currents, and tidal forcing is low (tidal range ~0.25m).

3.2.2. Sampling and data analysis

Two along-estuary surveys were carried out in Mahon on June 27 and October 24, 2011. Surface water samples (1–2 m depth) were collected using a peristaltic pump along a transect (15 stations) extending from the innermost area to an approximate distance of 1km outside the estuary (Figure 3.1). At each station, temperature and salinity profiles were obtained with a Seabird SBE-25 conductivity-temperature-depth (CTD). During the survey period, meteorological data were recorded by the local Port Authority, and water column temperature was fortnightly measured at station 10 as part of the monitoring program of the estuary. Current velocity data were obtained from two bottom-mounted acoustic Doppler current profilers (ADCPs) (1000 kHz Nortek Aquadopp profiler) deployed along the port axis, measuring at 1m depth intervals. The original 1-min currents were rotated along the local port axis direction and daily-averaged. Unfortunately, the ADCP in the middle part of the harbor failed at the end of September.

Nutrient analysis was carried out on water samples (13 ml) collected in acid-washed polyethylene tubes. Samples were pre-filtered through GF/F fiberglass filters (Whatman Ltd.), and then frozen until analysis. Concentrations of dissolved inorganic nitrogen (DIN = NO_2^-+ NO_3^-+ NH_4^+), soluble reactive P and silicate (Si), and total dissolved P were measured with an Alliance Futura autoanalyzer following Grasshoff et al (1983). In coastal waters soluble reactive elements provide a good approximation of the dissolved inorganic form for the correspondent element (Monaghan and Ruttenberg, 1999). Total dissolved P was estimated by pre-oxidizing the sample with an alkaline persulfate digestion that hydrolyzes all forms of inorganic and organic P to orthophosphate. DOP was estimated from the difference between total dissolved P and soluble reactive P. The accuracy of the analysis was established using Coastal Seawater Reference Material for Nutrients (MOOS-1, NRC-CNRC), resulting in 97%, 93%, 98%, and 118% for PO_4^{3-}, NO_3^-, NO_2^-, and SiO_2, respectively. The Limit of Detection (LOD), calculated as three times the standard deviation of subsequent blank measurements, was PO_4^{3-}: 0.05 μmol L^{-1}, NO_3^-: 0.001 μmol L^{-1}, NO_2^-: 0.001 μmol L^{-1}, NH_4^+: 0.03 μmol L^{-1} and SiO_2: 0.02 μmol L^{-1}. Chlorophyll a (Chla) concentrations were measured by fluorometric analysis of extracted pigments. Water samples (120 ml) were filtered through glass fiber filters (GF/F Whatman) and stored frozen. Pigments were extracted in 90% acetone and stored in the dark at 4°C overnight. The fluorescence of the extracts was measured on a Turner Designs fluorometer.

AP activity was assessed using the single cell ELF technique (Dyhrman and Palenik, 1999; Nicholson et al., 2006). Water samples (5 l) were pre-filtered through a 100 μm pore size mesh for zooplankton elimination, and then concentrated to 50 ml by inverse filtering through a 20 μm pore size Nytex filter. The sample was then gravity filtered onto a 20 μm filter. The filter was then transferred to an Eppendorf tube containing 1.5 ml of 70% ethanol. The tube was shaken to re-suspend the cells and the filter was removed. The resulting concentrate was incubated for 30 minutes, centrifuged at 2000 rpm for 15 min, and dried for 5 min. Finally 100 μl of a phosphomonoester substrate (ELF-97 endogenous phosphatase detection kit, Molecular Probes) were added to the sample, which was again incubated for 30 minutes. The ELF-97 substrate produces a yellow-green fluorescent precipitate at the site of ester hydrolysis, and thus labels any cells that express AP activity. Total microphytoplankton (hereafter phytoplankton) and ELF-labeled cells were enumerated with a Sedgewick Rafter chamber using a Leica epifluorescence microscope. Cells were identified using phase contrast illumination, and then examined with a 100-W mercury lamp and a long pass DAPI filter set (excitation 350 nm, emission 520 nm) for ELF activity. We estimated an average coefficient of variation for triplicate counts of 10% using this method.

3.2.3. P addition bioassay

A P addition bioassay was performed in situ during the June survey to test the response of the phytoplankton cells to P fertilization. The bioassay was designed to increase P_i in 0.08, 0.50 and 2.00 μmol L^{-1} through the addition of NaH_2PO_4 H_2O. Surface water from station 15 (Figure 3.1) was collected in 50 L acid-washed plastic carboys and pre-filtered through a 60 μm mesh to exclude large grazers. Incubations were performed in 6 L acid-washed PET bottles and consisted of triplicates for each P addition treatment and for control (no addition), resulting in a total of 12 incubations. The bottles were submerged at a depth of 1 m and incubated for 3 days. At the end of the incubation period, a sample was taken for each treatment to quantify phytoplankton abundance and taxonomy, and AP activity using the methods described previously. The experiment was carried out under clear skies with seawater temperatures oscillating between 24.5°C at night and 26.5°C at noon.

3.3. Results

3.3.1. Environmental conditions

Figure 3.2 shows the evolution of atmospheric and oceanographic conditions in the Mahon estuary during the study period. Atmospheric temperatures were in the range of 23-25°C in June, and declined to approximately 20°C by October (Figure 3.2A). Precipitation was typically low, with sporadic precipitation events. The evolution of seawater temperature revealed the development of a seasonal thermocline in late May to early June (Figure 3.2B). Surface seawater temperature progressively equilibrated with the atmosphere, while the thermocline deepened and intensified. In early October, the water column rapidly cooled down and became thermally homogeneous during the days previous to the October survey. Currents displayed a clear vertical pattern with two layers at the inner current profiler (ADCP1), whereas a more complex pattern occurred at ADCP2. For most of the study period, currents were predominantly directed seaward on the surface, and landward near the bottom, at ADCP1. However, this pattern reversed when the water column became thermally homogeneous in mid-October (Figure 3.2C). Three layers were observed at ADCP2 with landward bottom currents, seaward flow at mid depths (12-20 m) and variable on the surface, at least until the end of September when the ADCP2 failed (Figure 3.2D).

In accordance with this general flow pattern, the water column exhibited strong thermal stratification in the June survey. The thermocline was located at depths ranging from 14 to 18 m and presented a marked temperature gradient of ~2°C m^{-1} (Figure 3.3A). Slightly warmer and fresher waters in the inner estuary determined a net seaward flow of 1 to 2cm s^{-1} above the thermocline. The scarce precipitation, together with evaporation during summer, caused reversal of the longitudinal density gradient in the October survey, with cooler and saltier waters in the inner estuary (Figures 3.3B and D). In addition, an intense haline front in the estuary mouth separated inshore from offshore waters. Consistent with the reversal of the longitudinal density gradient, the only ADCP available in the October survey showed an inverse circulation with respect to the June survey.

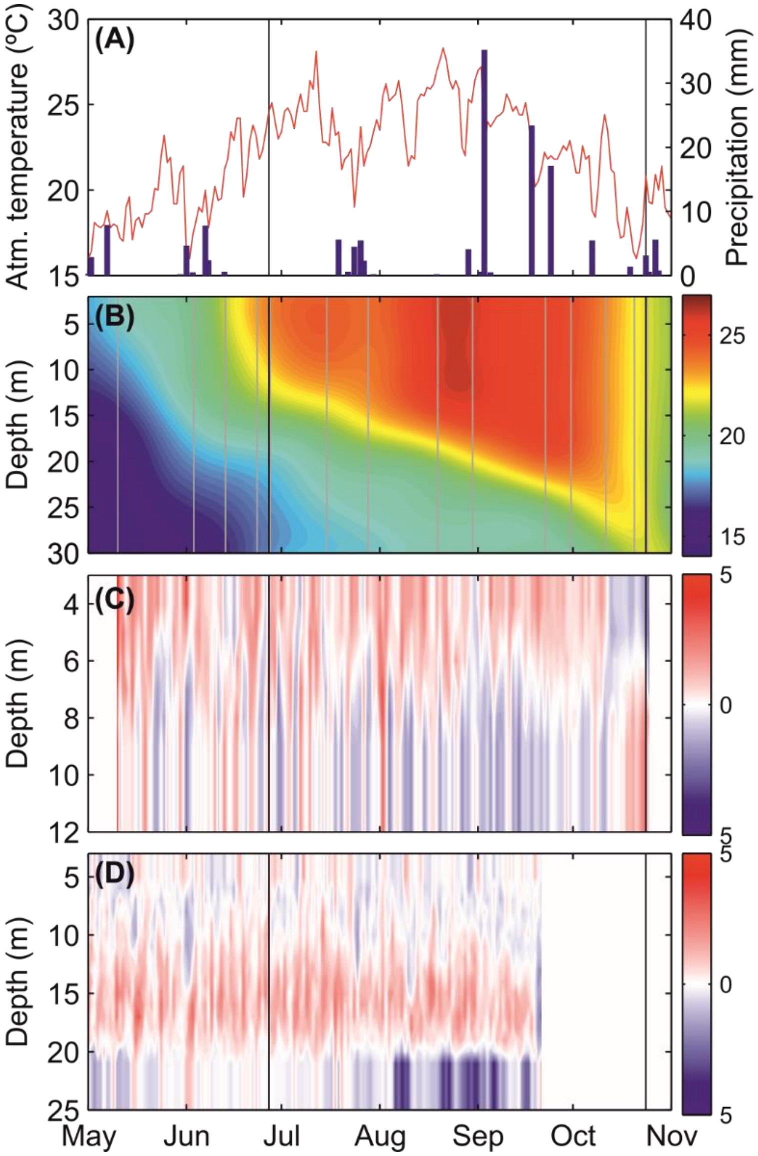

Figure 3.2 Time-series of (A) atmospheric temperature (°C, red line) and daily accumulated precipitation (mm, blue bars), (B) seawater temperature (°C) at station 10, (C) alongshore current (cm s-1) at the inner ADCP, and (D) alongshore current (cm s-1) at the middle ADCP. Vertical black lines indicate the survey dates (June and October) and both black and grey lines in (B) are the measured temperature profiles in the monitoring program. Blue and red color in (C) and (D) represent inshore and offshore direction, respectively

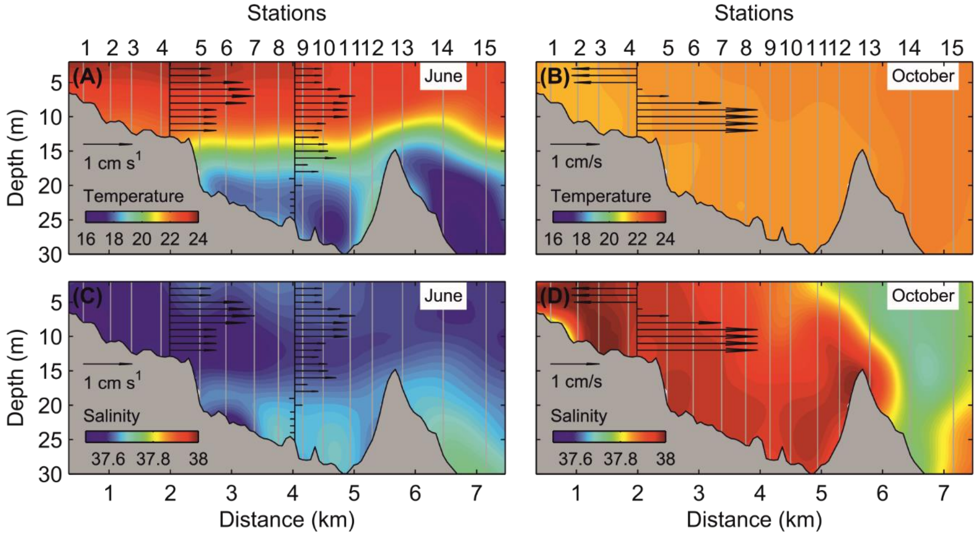

Figure 3.3 Along-estuary transect of: temperature (°C) for (A) June and (B) October surveys, and salinity for (C) June and (D) October surveys. The black arrows indicate the daily averaged axial currents (cm s-1) at different depths.

Figure 3.4 illustrates the variation of the measured nutrients along the estuary axis in both surveys. With some spatial differences, inorganic nutrients (DIN and P_i) exhibited enhanced concentrations in the inner estuary (stations 1-4), most likely because of the drains of Mahon town and its harbour. P_i was also enhanced at the proximity of the urban area of Es Castell, suggesting an additional urban source of nutrients in this area (stations 7-10; Figure 3.4C). Mean concentrations of DIN were slightly higher in June than in October (1.5 ± 1.53 and 1.0 ± 1.7 µmol L^{-1}). This was caused by the higher ammonium concentrations during the first survey (0.8 ± 0.44 and 0.2 ± 0.14 respectively). P_i was also slightly enhanced in June (0.09 ± 0.02 and 0.06 ±0.02 µmol L^{-1}, respectively). In contrast, DOP concentrations were significantly higher in the October survey (0.14±0.06 and 0.36±0.06 µmol L^{-1} respectively) and presented low spatial variations (Figure 3.4D). The SiO_2 showed a similar trend to that of DIN (Fig. 3.4E), with higher values in June than in October (1.7 ± 0.4 and 0.4 ± 0.1 µmol L^{-1} respectively). N:P molar ratios suggested potential P-limitation inside the estuary in both surveys (values above 16), although they covered most of the estuary (stations 1-7) in the June survey, and were restricted to the inner basin (stations 1-4) in the October survey (Figure 3.4F). Despite nutrient concentrations being higher, phytoplankton biomass, expressed as Chla concentration, was generally lower in June (mean 1.75±0.77 and 2.33±1.5 µg L^{-1}, respectively). Particularly enhanced Chla values (up to 5.1 µg L^{-1}) were detected in the inner estuary during the October survey.

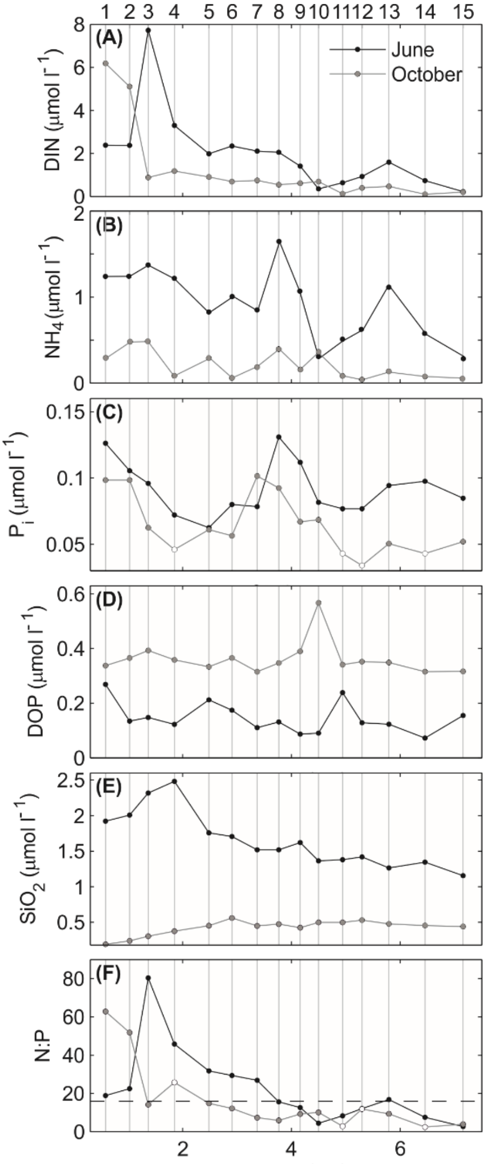

Figure 3.4 Surface (A) DIN, (B) NH4, (C) Pi , (D) DOP , (E) SiO2 , and (F) N:P ratio for June (black) and October (gray) surveys. The dashed back line in (E) indicates the Redfield ratio (16:1). White dots indicate values affected by the limit of detection (LOD).

3.3.2. Phytoplankton and ELF

The relative abundance of the main microphytoplankton species and their cell specific expression of AP as detected by ELF are shown in Table 3.1. During the June survey, the phytoplankton assembly was dominated by dinoflagellates accounting for 89% of the total abundance. *Prorocentrum* spp. (44%), *Scrippsiella* spp. (13%), and small unidentified dinoflagellates (16%) were the most abundant taxa. Conversely, the presence of diatoms characterized the phytoplankton community in the October survey, comprising 77% of the total cell abundance with *Chaetoceros* spp. (51%), *Cyclotella* spp. (14%), and *Asterionellopsis glacialis* (12%) being the most abundant taxa. Significant differences were also observed in ELF between both surveys. While 65% of total phytoplankton cell expressed AP in the June survey, this value decreased to 12% in October. However, AP was expressed almost exclusively by dinoflagellates in both surveys (99 and 98% of the total labelled phytoplankton abundance in June and October). *Prorocentrum* spp. (56%) was the most abundant taxa expressing AP in the June survey and small unidentified dinoflagellates (76%) in the October one.

Table 3.1 Total cell abundance and cells expressing ELF (cells l-1) for main phytoplankton taxa over the 15 stations during June and October surveys. Other taxa within diatom and dinoflagellate groups account for small unidentified species in the corresponding group. The percentage (%) of individual species to total cells and to group abundance is also indicated. The methodological error is 20% and the estimated counting error is ±10%. (ver anexos p. 192)

		Total		Total (%)				ELF- labeled		ELF (%)			
Group	Genus	Jun.	Oct.	Jun.		Oct.		Jun.	Oct.	Jun.		Oct.	
				% of total	% of group	% of total	% of group			% of total	% of group	% of total	% of group
Diatoms	*Asterionellopsis*	0	6862	0.0	0.0	12.0	15.5	0	31	0.0	0.0	0.5	23.8
	Chaetoceros	3932	29469	4.2	39.3	51.3	66.4	513	0	0.8	59.5	0.0	0.0
	Cyclotella	0	7919	0.0	0.0	13.8	17.8	0	31	0.0	0.0	0.5	23.8
	Licmophora	222	21	0.2	2.2	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Pseudo-nitzschia	208	26	0.2	2.1	0.0	0.1	0	21	0.0	0.0	0.3	16.2
	Rhizosolenia	439	10	0.5	4.4	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Striatella	739	0	0.8	7.4	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Thalassionema	901	0	1.0	9.0	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Thalassiosira	328	57	0.3	3.3	0.1	0.1	160	5	0.3	18.6	0.1	3.8
	Other	3230	42	3.4	32.3	0.1	0.1	189	42	0.3	21.9	0.6	32.3
Dinoflagellates	*Ceratium*	7140	373	7.5	8.4	0.6	3.2	2216	26	3.6	3.6	0.4	0.4
	Diplopsalis	2488	36	2.6	2.9	0.1	0.3	685	0	1.1	1.1	0.0	0.0
	Dinophysis	815	1575	0.9	1.0	2.7	13.6	197	204	0.3	0.3	3.0	3.0
	Prorocentrum	41711	840	44.1	49.3	1.5	7.3	35043	514	56.4	57.2	7.5	7.7
	Protoperidinium	4590	1155	4.9	5.4	2.0	10.0	3301	635	5.3	5.4	9.3	9.5
	Scrippsiella	12456	152	13.2	14.7	0.3	1.3	9281	105	14.9	15.1	1.5	1.6
	Other	15384	7448	16.3	18.2	13.0	64.3	10585	5221	17.0	17.3	76.3	77.8
Silicoflagellates		0	1423	0.0	0.0	2.5	100.0	0	10	0.0	0.0	0.1	100.0

The analysis of the spatial variations of phytoplankton community, Figure 3.5, shows the total cell abundances of the main phytoplankton taxa. In the June survey, both diatom and dinoflagellate taxa presented a marked along-estuary gradient although local enhancements were also observed for some taxa. Conversely, larger variations occurred in the October survey when diatoms dominated the phytoplankton community. The most abundant diatoms (*Chaetoceros* spp., *Cyclotella* spp., and *Asterionellopsis glacialis*) displayed maximum values in the middle of the estuary (stations 7-11). In contrast, other diatoms, most dinoflagellates and silicoflagellates, were distributed following the along-estuary gradient similar to the June survey.

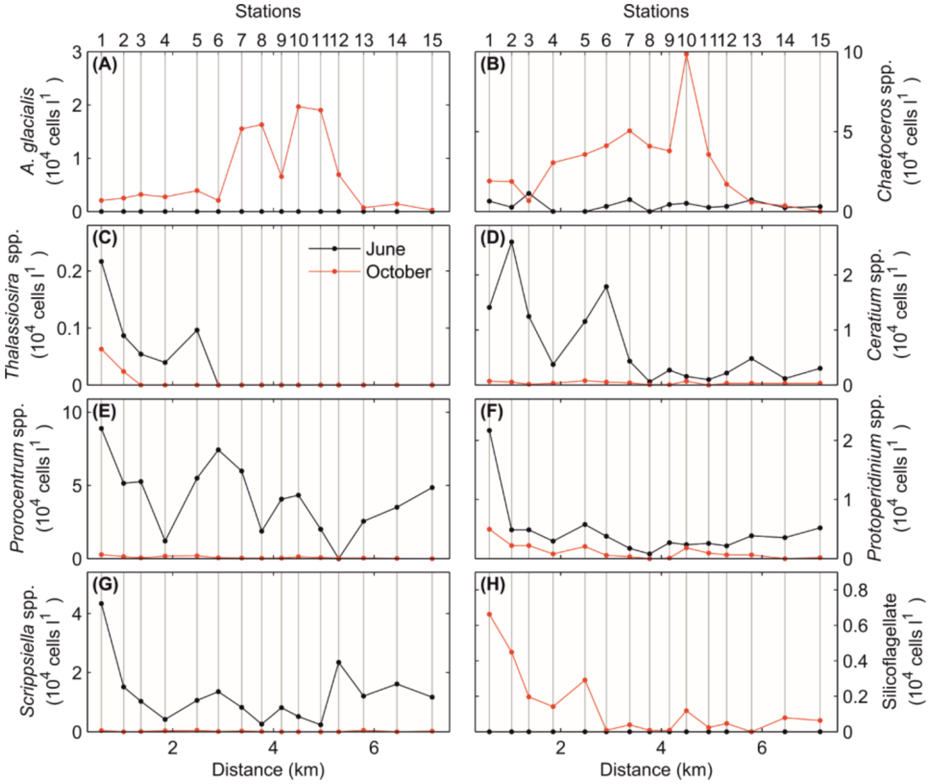

Figure 3.5 Total cell abundance (104 cells l-1) of (A) Asterionellopsis glacialis, (B) Chaetoceros spp., (C) Thalassiosira spp., (D) Ceratium spp., (E) Prorocentrum spp., (F) Protoperidinium spp., (G) Scrippsiella spp., and (H) Silicoflagellate for June (black) and October (red) surveys.

The along-estuary variations of the ELF-labeled cell percentages for main phytoplankton taxa are shown in Figure 3.6. As mentioned above, AP was mainly expressed by dinoflagellate taxa. Diatoms only expressed AP in relatively high proportion in the inner estuary during the June survey. This ELF signal was associated with species such as *Chaetoceros* spp. and *Thalassiosira* spp. As for the dinoflagellates, ELF for *Ceratium* spp., *Prorocentrum* spp., *Protoperidinium* spp., and *Scrippsiella* spp. followed the same along-estuary gradient as total cells in the June survey. In the October survey, the along-estuary gradient was less clear for *Ceratium* spp., *Prorocentrum* spp., and *Scrippsiella* spp.

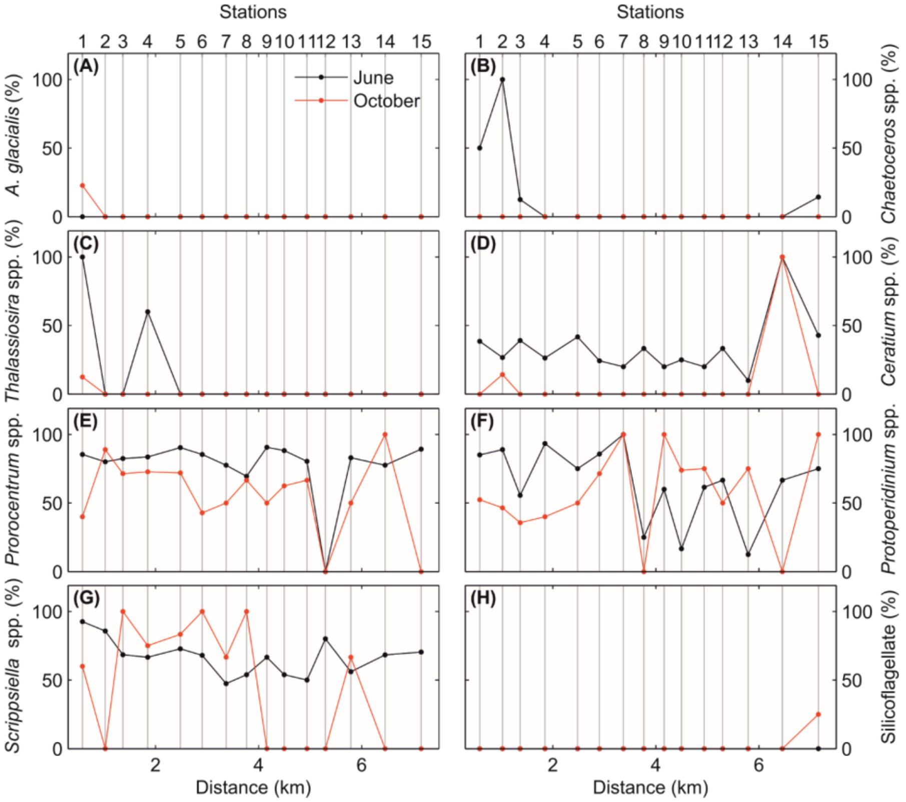

Figure 3.6 Percentage of ELF-labeled cells (%) of (A) Asterionellopsis glacialis, (B) Chaetoceros spp., (C) Thalassiosira spp., (D) Ceratium spp., (E) Prorocentrum spp., (F) Protoperidinium spp., (G) Scrippsiella spp., and (H) Silicoflagellate for June (black) and October (red) surveys.

3.3.3. P addition bioassay

The nutrient concentrations in the initial conditions for this bioassay were DIN 1.47 μmol L^{-1}, Pi 0.09 μmol L^{-1}, DOP 0.17 μmol L^{-1}. The phytoplankton community was dominated mainly by dinoflagellates (Figure 3.7), small unidentified dinoflagellates (42%), *Prorocentrum* spp. (19%), and *Dinophysis* spp. (13%). Diatoms, mainly small unidentified diatoms (8%) and *Pseudo-nitzschia* (4%), accounted for less than 16% of the phytoplankton community. Only 4 dinoflagellate taxa (*Prorocentrum* spp., *Protoperidinium* spp., *Scrippsiella* spp., and small unidentified dinoflagellates) expressed ELF. Figure 3.7 shows the total and ELF-labeled cell abundance for *Prorocentrum* spp., *Protoperidinium* spp. and *Scrippsiella* spp., and for *Dinophysis* spp. as example of species that did not express AP in the bioassay. Total cell abundance followed a similar trend decreasing with the addition of 0.08 μmol L^{-1}, and increasing with the additions of 0.5 and 2.0 μmol L^{-1}. The ELF-labeled cells in the control treatment always exceeded the 20% of the total for each taxon. This percentage rapidly responded to P additions decreasing to values <10%, even at the lowest P addition (+0.08 μmol L^{-1}). Also, ~5% of the total cells expressed AP even at highest P addition, suggesting that either some of the cells use other P sources even in the presence of high P_i concentrations and/or that the change in the metabolism of some dinoflagellate species is a slow process; *Protoperidinium* spp. was an exception to the general response to increased P addition. At the same time, the AP was expressed in almost 100% of the *Protoperidinium* spp. cells present.

3.4. Discussion

3.4.1. Environmental status

Our results reveal two contrasting hydrographic scenarios in the Mahon estuary. In the June survey, the water column was highly stratified, favored by the high heat flux during summer and the reduced tidal stirring. Moreover, the deeper water mass became isolated from the atmosphere, and also from the offshore waters due to the presence of a sill in the mouth of the estuary. The seaward flow of the surface layer resulted in a net, although weak, offshore transport. Water residence time was roughly estimated at some 18 ± 7 days, based on the assessment of freshwater replacement time. The physical setting in the October survey reveals a well-defined pattern characterized by weak vertical stratification and reversed estuarine circulation. In this scenario, longitudinal exchange due to buoyancy effects is expected to be weak, and the inverse structure could be considered as a result of long residence times (Largier et al., 1997; Nidzieko and Monismith, 2013). Based on the salinity gradient, the residence time was estimated to be 38 ± 14 days, suggesting strong decoupling between estuarine and offshore waters.

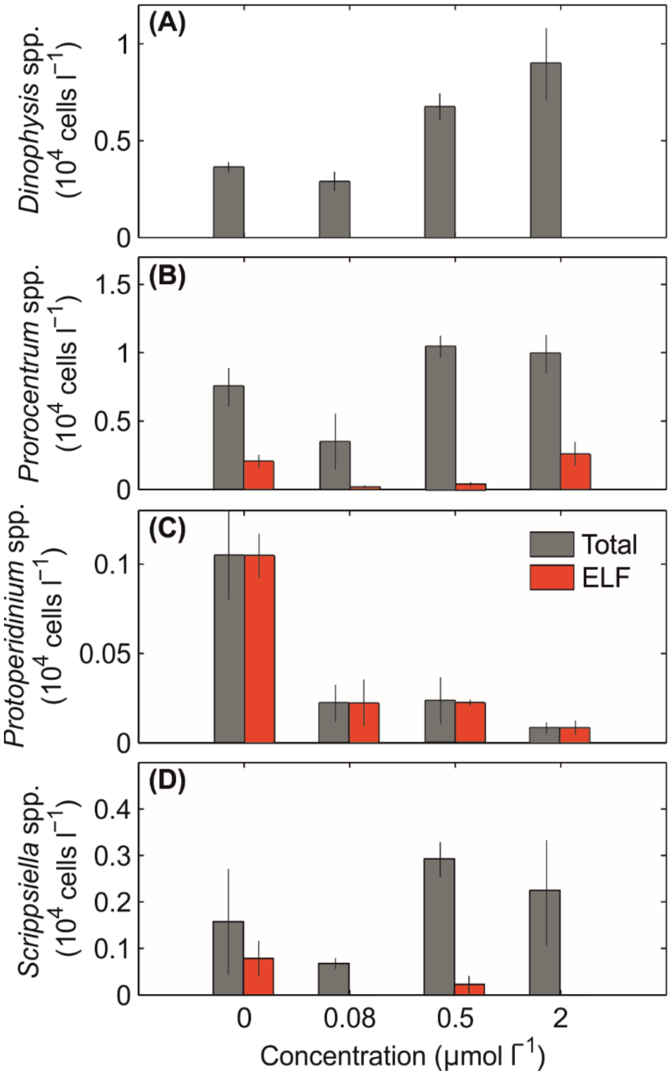

Figure 3.7 Mean (± S.D.) total and ELF-labeled cells abundance (104 cells l-1) of (A) Dinophysis spp., (B) Prorocentrum spp., (C) Protoperidinium spp., and (D) Scrippsiella spp. after three days of incubation for the control and Pi enriched treatments.

In spite of the different hydrodynamic situations, the inorganic nutrient concentrations displayed a relatively similar pattern in both surveys. The enhanced P_i concentrations in the inner estuary, and in the vicinity of Es Castell, suggest nutrient enrichment from terrestrial and/or anthropogenic sources (i.e. city mains, port activities). The June survey showed an overall higher DIN and P_i concentrations than that of October. Differences in DIN could be related to an increased ammonium level, which may be either indicative of intense zooplankton activity, or of reductive conditions in the deepest parts of the estuary (Figure 4B). Recovery of P_i levels may also be occurring via DOP remineralization. Strong top-down control by zooplankton explains the lower phytoplankton standing stock in this season. Contrastingly, a very different situation occurs in October, when biomass is enhanced (Chla 2.33±1.5 $\mu g\ L^{-1}$) but nutrient levels decreased. Differences in nutrients could be partially explained through sequestration in the phytoplankton standing stock. For example, assuming a P_i:Chla ratio of 1 $g\ g^{-1}$, differences of up to 0.04 $\mu mol\ L^{-1}$ could be justified by the differences in biomass between surveys. This would indicate an excess of inorganic nutrients and, possibly, a bloom prior to the October survey. Since rainfall was anomalously low in the weeks preceding the October survey, we attribute this higher nutrient availability to water column mixing, and to a sudden release of nutrients from the deeper layer caused by mixing. This process could bring nutrients, as well as organic compounds, from the isolated deep water mass to the surface, fuelling the phytoplankton growth.

3.4.2. *Species-specific P nutrition sources*

ELF characteristically forms precipitates at the site of AP activity, indicating which cells are responding to P limitation through the production of AP (González-Gil et al., 1998). Accordingly, our results reveal that at least part of the microphytoplankton community at Mahon presented some degree of P-starvation in both surveys. This is consistent with the elevated N:P ratios (above Redfield values) which also suggest P limitation. However, laboratory and field studies also reveal AP activity regulation by ambient phosphate concentrations (i.e. Cembella et al., 1984). While our bioassay generally confirms cessation of AP activity in most of the species in response to increased P_i, interpretation of ELF in natural conditions can be more challenging. For example, Mahaffey et al. (2014) suggest that while the phosphate concentration may have a first order control on the AP activity rates, other factors such as co-limitation by trace metals, influence the AP differences observed among oceanic regions. Differences in micro-nutrient requirements could also influence the species-specific response to ambient P_i concentrations.

In our case, we observed that P_i concentrations were slightly, though significantly, enhanced when AP activity was higher (0.09 ± 0.02 and 0.06 ±0.02 μmol L^{-1} in June and October, $p<0.05$). Several causes could explain this apparently contradictory fact (higher P_i and higher AP activity). P stress is defined as a physiological response to a low level of P_i, and the concentrations we measured are in the lower range of the values for marine systems (i.e. Conkright et al., 2000; Benitez-Nelson, 2000); particularly if we consider that the standing stock in Mahon is relatively high as compared with open Mediterranean waters. Therefore, the conditions both in June and in October could be similarly stressful. ELF labeling at these low P concentrations does not necessarily have to increase linearly with increasing Pi concentrations. In fact, competition with more efficient organisms, such as smaller phytoplankton cells (i.e. *Synechococcus*) and heterotrophic bacteria, which are abundant during this season (e.g. Agawin et al., 1998), may only leave a small proportion of ambient P_i available to larger microphytoplankton cells and thus forcing them to use organic P sources, which otherwise seem a rather available P-source. This also supports the idea that intracellular pools rather than ambient concentrations could be determining AP activity (i.e. Myklestad and Sakshaug, 1983; Gage and Gorham 1985). Finally, increased P_i in June could be in response to intense extracellular DOP hydrolysis driven by AP and other enzymes (nucleotidases, kinases or lyases) that release of orthophosphate ions to the environment (Ammerman and Azam, 1991).

It is found that AP activity was mainly expressed by dinoflagellates in both surveys. It is expected that different taxa would present diverse strategies for coping with P limitation, but even among the dinoflagellates, it is likely that there will be species with different physiological P status, despite being exposed to identical ambient concentrations of P_i and DOP (Mackey et al., 2007, 2012; Peacock and Kudela, 2013). Indeed, our bioassay reveals that even at the highest P_i concentrations some dinoflagellate species presented ELF. Plausible explanations are that either the dinoflagellates were P limited under those conditions, or that they had a slow regulatory response to P_i availability (e.g. Litchman and Nguyen, 2008). However, the generalized use of AP suggests a survival strategy in dinoflagellates to compensate for their less efficient competition for resources (Dyhrman and Palenik, 1999; Dyhrman and Ruttenberg, 2006). Other studies have also measured significant AP activity in high P_i systems (e.g. Nicholson et al., 2006), which questions the use of bulk AP activity as an indicator of P_i limitation.

In our bioassay, the addition of P_i resulted in a decrease of ELF in dinoflagellates such as *Prorocentrum* spp. and *Scrippsiella* spp., indicating a preferential use of P_i. Cessation in AP production has been explained in terms of energy cost of these enzymes (Chróst and Overbeck, 1987). Conversely, the production of AP in *Protoperidinium* spp. was a constant feature in the bioassay. The genus *Protoperidinium* is composed of some heterotrophic species known to perform extracellular digestion (Gaines and Elbrächter, 1987). The production of AP by heterotrophic dinoflagellate species is related to digestion and related metabolic processes (Skelton et al., 2006). Therefore, the expression of AP in many of *Protoperidinium* spp. cells observed in the bioassay could be indicative of cell catabolism rather than of DOP uptake.

The reasons behind the comparatively lower ELF in October could be attributed to different factors. There is a straightforward interpretation in relation to the change in species composition. Diatoms dominated the microphytoplankton assemblage during this survey and P status of diatoms is known to be more tightly controlled by P_i availability than it is for dinoflagellates (Dyhrman and Palenik, 1999; Nicholson et al., 2006; Dyhrman and Ruttenberg, 2006). However, this does not by itself explain the lowered AP activity because flagellates also presented reduced AP. As discussed in the precedent section we suggest that the higher phytoplankton biomass in October was caused by an increase in the nutrient availability and growth during the days prior to the survey. In other Mediterranean estuaries, P_i buildup has been observed during hypersaline conditions associated with heterotrophic decomposition of particulate organic matter (Christie, 1981; Largier, 1997). Phytoplankton may display luxurious feeding when nutrients are readily available and contain sufficient internal stores of P to avoid synthesizing AP (John and Flynn, 2000). Under these conditions diatoms could be competitively favored because of their faster growth and nutrient uptake rates. In fact, diatoms present in the October survey were amongst the lowest cells expressing AP. Without excluding seasonal variations in plankton composition and metabolism such substitution of *Synechococcus* by the less P-efficient *Prochlorococcus* cells at the end of summer, or decrease in heterotrophic bacteria activity at lower temperature, could also favor the availability of a larger P_i pool to microphytoplankton in this survey.

3.5. Conclusion

Our results show that the interaction between hydrography and nutrient sources played a prominent role in the regulation of plankton communities in the Mahon estuary, which may ultimately alter the entire plankton trophodynamics. Also, while preference for P_i in most species is acknowledged, both organic and inorganic P-pools sources are exploited by the different groups of microphytoplankton possibly due to internal and external factors. This yields a wide variety of species-specific strategies to satisfy the nutritional requirements. Indeed, our results show that ELF did not indicate generalized P-deficiency in the phytoplankton community of Mahon. Various factors may regulate AP activity, such as internal P storage, or the composition of the phytoplankton community, as well as the specific physiological requirements. This should be taken into account when interpreting AP as P-deficiency indicator, particularly in the case of some dinoflagellate species which use DOP even when P_i is relatively high. In addition, nutritional modes of particular species such as heterotrophy also need to be considered. Therefore, it is important to assess the whole phytoplankton community, at genus/species level, in studies of either organic or inorganic P uptake capabilities.

Acknowledgments

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Capítulo 4

Caracterización geoquímica de los sedimentos del puerto de Mahón (Menorca isla, España): una evaluación de la contaminación potencial por metales. / Geochemical characterization of sediments of the Mahon harbour (Minorca island, Spain): an assessment of potential pollution by metals.

Este capítulo será enviado a publicar en:

- Tovar-Sánchez, A., Martínez-Soto, M. C., Sánchez-Quiles, D., & Basterretxea, G., (En Preparación). Geochemical characterization of sediments of the Mahon harbour (Minorca island, Spain): an assessment of potential pollution by metals. *Science of the Total Environment.*

Abstract

Mahon (Minorca) is narrow and semi-enclosed harbour impacted by historic urban and industrial metal pollution. We analyse and compare surficial and long-term sediment records along the Harbour to assess the sources and historical trends in pollution. Trace metal concentrations obtained in sediments of Mahon Harbour were of the same order of magnitude than those measured in other anthropogenically polluted coastal areas in the Mediterranean Sea. Only Hg presented significantly higher concentrations in the harbour than compared sites (up to 2 orders of magnitude higher). Although surficial concentrations of some metals (such as Hg, Pb, Cu and Zn) are were lower than old in the historic sediment record, they showed levels above the recommended for acceptable sediment quality. Enrichment factors (EF) calculations confirm the anthropogenic origin of metals (e.g. Hg and Pb), linked to

the history of growth of the industries in the Mahón harbour. However, some variations can be attributed to natural fluctuations in the sediment deposition in the harbour. Meanwhile, the dysoxic conditions in depth sediments, suggest the polluting potential that may have these sediments if are exposed to the surface.

4.1. Introduction

Marine sediments are defined as aggregates of countless numbers of insoluble particles of unconsolidated material, which have been transported to the bottom of the oceans and seas by various transport agents (Lutgens and Tarbuck, 1986). In many cases these sediments, which are the ultimate repository of most of the waste generated by man in coastal areas, contain information about the events that occurred in preindustrial time in the bays and their catchment area and can thus be used as sensitive indicators for monitoring the spatial and temporal distribution of pollutants. Changes in metal concentration with depth (time) in sediment cores can show long-term trends of metal inputs and thereby pollution episodes (Balls et al., 1997; Kishe and Machiwa, 2003). Vertical profiles of pollutant species in sediment cores have been commonly used as "pollution records", providing information on the current system and the geochemical changes that occur over time in these environments. Chemical composition of sediment cores can be used to establish background conditions and to evaluate how contaminant levels have responded to changes in population, land use and human activities (Bonilla et al., 2003; Dassenakis et al., 1997; Rubio et al., 2000; Tuncer et al., 2001).

Trace metals, from natural or anthropogenic origin, are deemed serious pollutants because of their toxicity, persistence, and non-degradability in the environment (Fang and Hong, 1999; Klavinš et al., 2000; Tam and Wong, 2000; Yuan et al., 2004). Moreover, these elements accumulated in sediments could be a potential source of toxicity to the ecosystems they are connected with (Buccolieri et al., 2006; Glasby et al., 2004; Maanan et al., 2004). Trace metals are transported to the marine ecosystem through rivers, run-off, atmospheric transport and local sources (Glasby et al., 2004; Maanan et al., 2004). Thus, mobilization rates from anthropogenic sources sometimes equal or exceed natural fluxes (Bruland et al., 1974). Numerical sediment quality guidelines (SQGs) are nowadays widely used to identify and assess the contamination degree of sediments because they are based on the biological effects in freshwater or marine ecosystems (Long et al., 1995; MacDonald et al., 2000). Most of the studies on the concentrations of metals in the surficial sediment and biota are based on the determination of the bulk of trace metal concentration and comparing them with background or reference values (Breslin and Sañudo-Wilhelmy, 1999; Chapman and Mann, 1999).

Harbours are enclosed and low-energy water bodies where fine-grained sediment tends to accumulate. Their activities are diverse and, therefore, they represent marine ecosystems susceptible to receive significant metal inputs from marinas, boat hull maintenance, wholesale fish markets, shipping activities, urbanization, tourism, agriculture, marine traffic, industrial activities, storm and urban waste discharges, etc. These activities lead the harbour system to be severely altered with high environmental pressure and a gradual alteration of water and sediment quality (e.g., Ahumada, 1995; Casado-Martínez et al., 2005; Lafabrie et al., 2007; McCready et al., 2003; Rudolph et al., 2002; Sprovieri et al., 2007; Tovar-Sánchez et al., 2010; Usero et al., 2005).

The port of Mahon (Minorca Island, Spain) with a length of 5 km, a width of 1.2 km and a depth of up to 30 m, is considered to be the largest natural harbor in the world. This characteristic, coupled with its location in the Mediterranean Sea, has made it a strategic stronghold for many nations throughout its history (from Roman to British). Today, Mahon harbour is a modern port in natural and technically equipped surroundings, counting with a complete sector of repairs and mechanical navy factories. The exceptional characteristics of the port of Mahon, its history and its geographic location makes it a place with high touristic impact. Current commercial activities established around the harbor are represented by cheese, shoe, jewellery, metallurgy and thermal electricity industries, tourism, aquatic sports, etc. The port of Mahon is sheltered from coastal currents and has poor water renewal due to its micro-tidal regime, which makes it difficult to disperse urban or industrial discharges into the open sea. This in turn allows the establishment of progressive sediment contamination by metals inside the port (Garcia-Orellana et al., 2011). This study presents the geochemical characterization of sediments in Mahon harbour with the aims to assess their trace metal levels of contamination.

4.2. Materials and Methods

Surface sediment samples (1-2 cm) and core sediments (up to 55 cm depth) were collected by a scuba diver in June 2010 and July 2011, respectively, in 10 stations located along the Mahon Harbour (Figure 4.1). Since the outer to the inner harbour where the sediment is prone to be affected by re-suspension and the anthropogenic impact. Surface sediment samples were collected and stored in polyethylene test tubes at stations S-1 to S-6 (Figure 4.1), whereas sediment cores (stations C-3, C-4, C5 and C-7) were collected with the aid of methacrylate cylinders (100 cm i.d.), sliced into 1-cm sections and then placed in polyethylene test tubes. All sediment samples were stored frozen in polyethylene bags until processed in the laboratory.

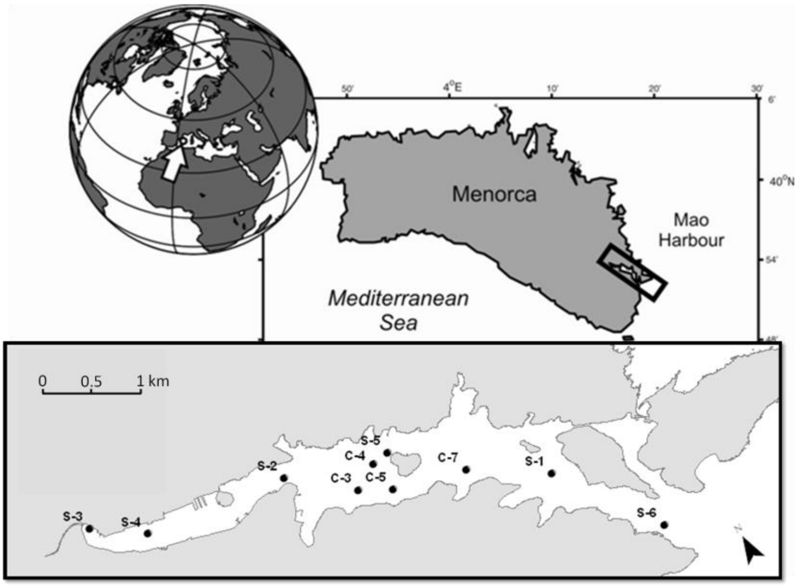

Figure 4.1 Study site showing the Western Mediterranean, the Minorca Island and the Port of Mahon with sampling stations.

Radiometric analyses were carried out in the bulk fraction at the Laboratori de Radioactivitat Ambiental of the Universitat Autònoma de Barcelona. Determination of total ^{210}Pb activities was accomplished through the measurement of its alpha-emitter daughter nuclide ^{210}Po, following the methodology described in Sanchez-Cabeza et al., (1998). After addition of ^{209}Po as an internal tracer supplied by International Atomic Energy Agency (IAEA) (0.7031 ± 0.050 Bq ml^{1}), sample aliquots of 200-300 mg were totally digested in acid media by using an analytical microwave oven and Po isotopes plated on silver discs in HCl 1 N at 70 °C while stirring for 8 h. Polonium emissions were subsequently counted for 1-4x10^{5} s with α-spectrometers (EG&G Ortec) equipped with low background silicon surface barrier (SSB) detectors. Chemical recoveries ranged from 85% to 100% and energy resolutions from 20 to 35 keV. The activities of ^{137}Cs and ^{226}Ra were determined by γ spectrometry in a coaxial high-purity Ge detector (EG&G Ortec) calibrated with the SRM-4276 standard solution (National Institute of Standards and Technology). Samples were sealed at least 3 weeks prior to counting to allow equilibrium between ^{226}Ra and its short-lived daughters. The ^{226}Ra activity was determined from ^{214}Pb through its 351 keV gamma line emission. Excess ^{210}Pb (^{210}Pb$_{xs}$) was determined by subtracting the ^{226}Ra activity (assumed to represent the supported ^{210}Pb activity) from the total ^{210}Pb activity. Quality of the measurements was assessed by the laboratory participation on IAEA proficiency tests and the continuous analyses of certified reference materials and replicates.

Quasi-total metal concentrations (Al, Cu, Co, Cr, Fe, Mn, Ni, Pb, Zn, Ca and Sr) were determined by ICP-OES (Perkin Elmer ICP-OES Optima 5300 DV). Samples were dried in an oven at 60 °C for two days and subsequently grinded in a zirconia ball mill (10 minutes at 170 rpm) before analysis. Metals were extracted with a microwave acid digestion system (CEM model Mars 5) according to the SW-846 EPA Method 3051A (Lyman et al., 1987), which involved the digestion of 0.2 g of sediment sample by triplicate with 10 ml of nitric acid (65%, Suprapur quality) in Teflon vessels. After the digestions the samples were diluted to 50 ml using Milli-Q water and then analysed. For the specific case of Hg, their concentrations were determined by a Direct Mercury Analyser (Milestone DMA-80). The accuracy of the analysis was checked with the certified reference material PACS-2 (National Research Council Canada), with recoveries that ranged from 70 to 100% for all metals analysed. Detection limits, were calculated as three times the standard deviation of the blank values.

Grain-size analysis was carried out by passing wet sediment samples through a 63 µm sieve after treatment of the samples with hydrogen peroxide. The percentage of sediment fraction finer than 63 µm was determined using a laser particle size counter COULTER-LS, with a resolution of 100 channels between 0.4 and 1000 µm. Weight percentages of sand (63 µm), silt (63–4 µm) and clay (4 µm) were recalculated and normalized to 100%. Mean sediment size was also used for a better resolution of grain-size fluctuations. Mean mud size (X_a), which represents the average size of all the particles in one sample, was calculated from the expression $X_a = S (X_{c \cdot nc})/S_{nc}$, where X_c is the median size of the channel (in µm) and nc is the percentage of particles in each channel.

A chemical sequential extraction scheme (Huerta-Diaz and Morse, 1990) was carried out to obtain the operationally-defined Fe fractions HCl and pyrite, which were subsequently used to determine the degree of pyritization (DOP; Berner, 1970). We used a predetermined amount of homogenized wet sediment equivalent to 2.5 g dry weight, which was previously calculated from the percentage by weight of water and solid for each sample. Sediments were digested during 16 hours with 20 ml of 1 N HCl to obtain the HCl fraction (which includes carbonates, Fe and Mn oxides, and acid volatile sulphides, or AVS). The silicate fraction (aluminosilicates, mainly clays) was obtained by two successive digestions using, each time, 30 ml of 10 M HF (1 and 16 hours, respectively). Finally, the pyrite fraction was obtained by dissolving the resulting residue with 10 ml of concentrated HNO_3 for one hour. Since the silicate fraction is associated to non-reactive trace metals and is not important during the processes of early diagenesis (where pyrite is formed), it was not considered in the analysis (Huerta-Diaz and Morse, 1990).

4.2.1. Data analyisis

The degree of pyritization (DOP) of the sediments was calculated by combining the Fe concentrations in the HCl and pyrite fractions (i.e., Fe_{py} and Fe_{HCl}, respectively). This term was introduced by Berner, (1970) and used to evaluate the amount of Fe present as pyrite relative to the so-called "reactive Fe" (Fe_{py} + Fe_{HCl}). The DOP was calculated using the following equation:

$$DOP(\%) = (Fe_{py} \ / Fe_{HCl} + Fe_{py}) \times 100 \qquad (1)$$

Organic Carbon concentrations were determined with a Carlo-Erba EA1108 Elemental Analyser with an average uncertainty of 1%. Prior to analyses, all samples were treated with HCl 2 N to remove carbonates, rinsed using Milli-Q water and dried at 60 °C (Kennedy et al., 2005).

We used two methods to estimate the influence of the anthropogenic activities or the degree of contamination in the surficial and core sediments of Mao Harbor. First, by calculating enrichment factors (EF) relative to average values of the continental crust (Wedepohl, 1995). Normalization of our trace metal concentrations was made with Al to minimize the effects of grain size, a procedure that has been widely used for evaluating metal enrichments in estuarine and coastal sediments (e.g., Abrahim and Parker, 2008; Covelli and Fontolan, 1997; Ho et al., 2010; Rubio et al., 2000). Enrichment factor (EF) values were calculated as:

$$EF=((C_x/C_{Al}))sample/((C_x/C_{Al}))background$$

where C_x and C_{Al} are the concentrations of the trace metals and aluminium measured in sediment samples and in background, respectively. The anthropogenic influence can then be assessed with the calculated EF values using the seven contamination categories of Dung et al. (2013): $EF \leq 1$: no enrichment, $1 < EF \leq 3$: minor enrichment, $3 < EF \leq 5$: moderate enrichment, $5 < EF \leq 10$: moderately severe enrichment, $10 < EF \leq 25$: severe enrichment, $25 < EF \leq 50$: very severe enrichment, $EF > 50$: extremely severe enrichment.

We also calculated the geoaccumulation index (Igeo) to estimate the degree of sediment contamination. This index was originally used by Müller, (1986) and it has been widely used (e.g., Buccolieri et al., 2006; Covelli and Fontolan, 1997; Ho et al., 2010; Loska et al., 1997; Ruiz, 2001; Santos Bermejo et al., 2003) to define the degree of metal contamination in sediments by comparing a given concentration with the preindustrial levels, It can be calculated as follows:

$I_{geo} = \log_2 * [C_n / 1.5B_n]$

where C_n is the measured concentration of the relevant metal in the sediment and B_n is its background concentration. The factor 1.5 is used to compensate for possible variations in background levels for a given metal in the environment and also already very low anthropogenic pressures. Similarly to the case of the EFs, we used the concentrations reported by Wedepohl, (1995) as background values. The Müller classification distinguishes seven Igeo classes: unpolluted ($Igeo \leq 0$); unpolluted to moderately polluted ($0 \leq Igeo < 1$); moderately polluted ($1 \leq Igeo < 2$); moderately to strongly polluted ($2 \leq Igeo < 3$); strongly polluted ($3 \leq Igeo < 4$); strongly to very strongly polluted ($4 \leq Igeo < 5$); very strongly polluted ($Igeo \geq 5$).

In Spain, Action Levels (named AL1 and AL2) are used to characterize dredged material (AL; CEDEX, 1994) and represent hazardous concentrations for marine organisms based on physicochemical criteria. When the measured concentrations are below AL1, the dredged material can be discharged directly into the sea; when the values of the contaminants are between AL1 and AL2, additional investigations are required and the discharge should be monitored. Finally, when pollutant values are higher than AL2, the dredged material should be isolated.

4.3. Results and Discussion

4.3.1. Surficial Sediments

Some of trace metals presented here have already been described and discussed in Martinez-Soto et al., 2016. Metal concentration results (Al, Ca, Sr, Co, Cu, Fe, Hg, Mn, Ni, Pb and Zn) from surficial sediments of Mahon Harbour (Table 4.1) showed that Ca, Al and Fe varied between 8 - 26%, 0.2 - 2.9%, and 0.2 – 2.3% respectively. The rest of trace metals varied (in $\mu g\ g^{-1}$) between 64 - 234 (Mn), 1 – 22 (Ni), 1 - 6 (Co), 14 - 124 (Pb), 17 - 134 (Zn), 2 - 81 (Cu) and 0.04 - 1.43 (Hg). Added to this, the dominant grain sizes present in most of the surfaces samples were sand and silt, varying between silty-sand, sandy-silt and silt (Table 4.1).

Table 4.1 Average metal concentrations ± one standard deviation (SD), grain-size and total organic carbon (TOC) in surficial sediments from Mahon Harbour. Metal concentrations are expressed in µg g-1, except Ca, Al, Fe and TOC that are in % (dry weight). (ver anexos p. 193)

	Ca (%)		Sr		Al (%)		Fe (%)		Mn		Ni		Co		Pb		Zn		Cu		Hg		TOC (%)	Grain size
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD		
S-3	7.9	0.5	419	16	0.8	0.2	1.0	0.0	106	4	9.7	0.6	3.3	0.5	42	1	93.5	0.4	75.2	1.2	0.30	0.01	1.1	Sandy-silt
S-4	11.2	0.9	603	122	1.8	0.1	1.8	0.0	172	4	16.4	0.2	5.7	0.2	124	3	77.2	1.1	40.8	0.7	1.43	0.04	0.8	Sandy-silt
S-2	17.9	1.0	1127	168	0.7	0.0	0.9	0.0	79	2	7.6	0.3	3.0	0.2	56	2	67.2	4.2	33.4	1.6	1.16	0.82	1.7	Sandy-silt
C-3	10.8	0.3	848	22	2.2	0.1	2.2	0.1	222	8	20.6	0.8	6.0	0.3	104	4	125.4	4.1	72.2	2.7	1.11	0.04	2.8	Silty
C-4	14.1	0.3	1068	19	2.4	0.0	1.9	0.0	209	2	16.4	0.2	5.3	0.1	89	2	111.6	3.4	59.0	1.3	0.87	0.04	2.2	Silty
S-5	26.3	1.0	1693	35	0.2	0.0	0.2	0.0	64	2	1.4	0.6	0.9	0.1	14	2	17.1	0.7	2.1	0.2	0.04	0.00	0.4	Silty-sand
C-5	11.6	0.2	879	13	2.1	0.1	2.2	0.1	225	5	21.2	0.3	6.0	0.3	109	1	131.1	2.5	77.2	4.3	1.22	0.01	2.9	Silty
C-7	13.6	0.3	1019	13	2.3	0.1	2.2	0.0	226	3	5.8	0.3	20.9	0.4	112	5	125.5	2.8	71.4	1.2	1.01	0.04	2.6	Silty
S-1	14.1	0.2	1048	15	1.8	0.3	1.6	0.0	181	5	16.2	0.3	4.5	0.4	93	6	91.2	1.8	48.0	0.5	0.84	0.03	1.6	Silty-sand
S-6	10.1	0.1	768	2	2.2	0.2	2.0	0.0	207	1	19.8	0.7	5.6	0.0	113	3	122.6	3.2	71.5	2.2	1.22	0.00	1.8	Silty-sand

Av: average SD:standard desviation

Iron, Mn, Ni and Co concentrations in surficial sediments presented similar distributions (Figure 4.2), with high concentrations in locations with fine grain-size (sandy-silt and silt; S4, S6, C3, C4, C5 and C7) and low concentrations at S3, S2, S1 and S5 where the predominant type of sediment is sandy. It is generally accepted that high trace metal concentrations are associated to fine grain size (silt) sediments. This could be due to the organic matter accumulation in the silty sediment (see Table 4.1), since trace metals have affinity for organic matter. Calcium and Sr showed an opposite behaviour to Fe, Mn, Ni and Co, with the highest concentrations at sandy sites (Figure 4.2). Calcium and Sr are considered lithophile elements that remain on or close to the surface because they combine readily with oxygen and do not have affinity for organic matter. Also, it is known the Ca and Sr affinity for carbonate formation; therefore, they can be associated to calcareous remains in the coarse sediments (sand). The rest of the elements (i.e., Hg, Pb Cu, Zn) show trends similar to Fe, Mn, Ni and Co, with the exception of the inner harbour sediments (i.e., S-3, S-4 S-2), where a clear trend was not observed, probably because their concentrations are controlled by local processes.

As discussed in Martinez-Soto et al., 2016 some metal concentrations measured in surficial sediments of Mao Harbour are similar to those reported for other Mediterranean coastal areas impacted by human activities. Meanwhile Hg, showed enriched concentrations (between 40 – 98%) than other Mediterranean coastal areas (Buccolieri et al., 2006; Lafabrie et al., 2007; Romano et al., 2004). Therefore, we can suggest that, Mahon harbour is a coastal area a with particular anthropogenic pressure. These high levels of Hg and the other trace metals mentioned at Martínez-Soto et al., (2016), could be the result of the impact of the industrial activities around the harbour, as previously suggested by (Garcia-Orellana et al., 2011).

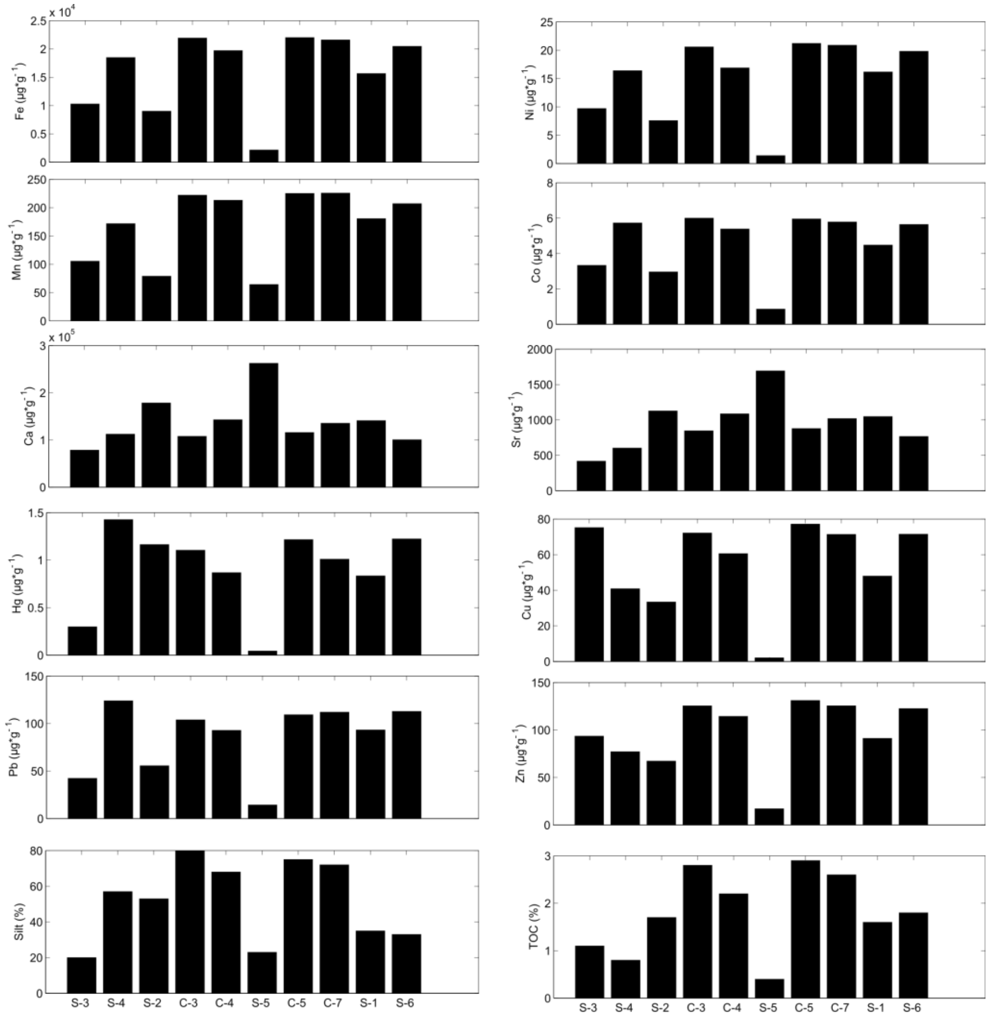

Figure 4.2 Surficial sediment concentrations of Fe, Mn, Ni,Co Ca,Sr, Hg, Pb, Cu and Zn (µg g -1) , TOC (%) and Silt content (%).

4.3.2. Sediment Cores

The length of the collected sediment cores, which ranged from 22 cm (C-3) to 55 cm (C-5) provides information from the 1890's up to the present. Hence, metals in the Mahon sediment column will reflect the history of industrial activities developed in the area since the mid-19th century. All collected cores presented silt + clay textural characteristics, containing between 70 (core 7) and 100% (core 5) of the fraction < 63 µm.

Concentrations in the sediment cores varied from 8124 to 31769 µg g^{-1} for Fe, 77 to 356 µg g^{-1} for Mn, 8 to 30 µg g^{-1} for Ni and 2 to 10 µg g^{-1} for Co. Interestingly, concentrations of these metals increased from the end of the 19th century until 1960's in all cores, a characteristic that is probably related to the beginning of industrial activities in the Harbour. During the mid to late 19th century some industrial activities developed around the harbour, such as marine, motor, textile, jewelry and shoe industries, which began to become important. Industry development was followed by upgrading of the primary sector in subsequent decades, despite the presence of economic problems in the first decades of the 20th century and the postwar years, as suggested by the slight increase in Fe, Mn, Ni and Co concentrations in the sediment record. After the 1950s, Mahon and its harbour experienced an increase of industrial activities, mainly jewellery and footwear (López Casasnovas, 1991; Riera Sastre, 2005). The recovery of the economy was recorded in the sediments with a higher increase of Fe, Mn, Ni and Co concentrations until 1960's.

The Ca and Sr profiles showed an opposite trend with respect to The Fe, Mn, Ni and Co concentration profiles (Figure 4.3) showed the highest values between 1890 and 1960, ranging from $5x10^3$ to $23x10^3$ µg g^{-1} for Ca, 503 to 1999 µg g^{-1} for Sr (concentrations in µg g^{-1}). Before the 1960, the content of Ca in sediments was high, probably due to presence of carbonate algae communities in the Harbour (Rodriguez Femenias, 1889; Siguan and Garreta, 1985). In the 1950s the prosperity in the industrial production and an increase in port activity caused changes in the dynamic and physico-chemical conditions of Harbour waters, altering the marine environment and probably causing the mortality of many algae species and consequently the reduction of Ca and Sr content in sediments. In the same way that the rest of the elements Ca and Sr concentrations remain stable from 1970, probably as consequence of the controlled discharges to the Port.

Mercury, Pb, Cu and Zn showed higher concentrations than other coastal zones with anthropogenic impact, with concentrations ranging from 0.1 to 2.6 µg g^{-1} for Hg, 17 to 202 µg g^{-1} for Pb, 6 to 196 µg g^{-1} for Cu and 26 to 173 µg g^{-1} for Zn. These results suggest that Mahon Harbour might have had a strong anthropogenic influence starting in the early twentieth century (Figure 4.3). Mercury, Pb, Cu and Zn probably had different sources from several industrial activities that settled in the Port. For example, Pb and Hg were used as a pigment in textile industry, which settled in the region in 1902 (Nagajyoti et al., 2010) and were present in leaded gasoline between 1920 and 1989 (Conaway et al., 2005; Won et al., 2007). Coal burning can produce Zn

(Bhangare et al., 2011; Vassilev, 1994) and Cu can be present in fertilizers, pesticides, decorative ceramics, blue pigments used in the textile industry and as a component of antifouling paint (Nagajyoti et al., 2010).

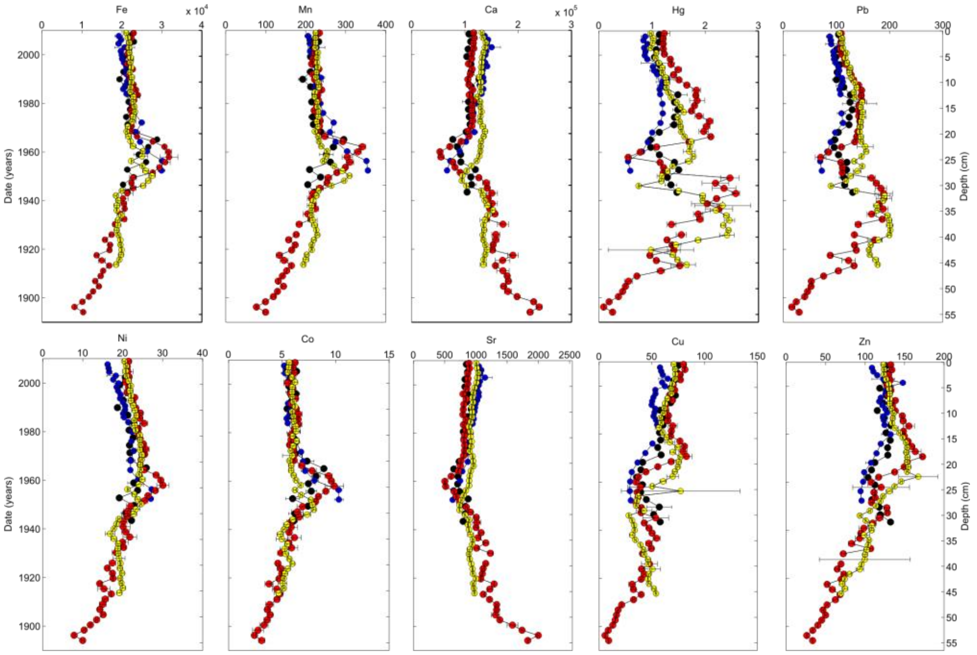

Figure 4.3 Trace element concentrations in the sediment cores collected in Mahon Harbour. Black, blue, red and yellow symbols represent cores C-3, C-4, C-5 and C-7, respectively. Error bars represent standard deviation. All concentration are in µg g -1 dry weight.

Fluctuations in Hg, Pb, Cu and Zn concentrations observed between the 1920s and 1970s probably are due to changes in activities that occurred in the different industries located around the Mahon Harbour. Despite the economic problems in the first decades of the twentieth century, caused by the First World War, from the decade of the 1920s there was a Menorcan business resurgence (López Casasnovas, 1991; Riera Sastre, 2005) during which the industry of silver purses took a turn doing the conversion to jewellery, several furniture companies were opened and footwear remained at a good economic level. These changes in economic activity are probably reflected in changes in the quantity and type of metal accumulated in the sediments.

So it makes sense that Hg, Pb, Cu and Zn concentrations were increased until 1925 and continued to remain roughly constant until 1940.

After the period 1936-1939 and post-war years (a delicate time for Menorcan economy), occurs a slow business reactivation process in the 1950s under the liberalization of economic policies of the regime (López Casasnovas, 1991; Riera Sastre, 2005). The footwear industry kept their particular career line, jewellery reappeared, furniture, electronic components business (Riera Sastre, 2005). It is then when there was a reactivation of the cheese industry and Quesera Menorquina (COINGA), opened in 1966, was one of the most important industries in the region (Riera Sastre, 2005). All this resulted in prosperity in the 1950s and 1960s, especially in the production of jewellery and shoes. These activities were recorded in sediments with variations of Hg, Pb, Cu and Zn concentrations between 1950s and 1960 and subsequent slight increases until the 1970s. In Menorca tourism showed a slowdown, possibly due to the consolidation of this activity, Mahon Airport received its first international flights in 1969. The construction of a sea outfall pipe in 1978, in order to evacuate the waste of jewellery factories and urban wastes out of the harbour, also allowed the reduction of the concentration of contaminants in harbour waters and sediments, resulting in a continuous slight decrease and stabilization of the Hg, Pb, cu and Zn content in sediments from the 1980s until now.

To distinguish the influence of the different industrial activities on the sediment metal distributions as a function of time in the Mahon harbour, a Principal Component Analysis (PCA) was performed (Figure 4.4). Our results show that the two first components account for 93% of the variance (components 1 and 2 represent 81 and 12%, respectively). The first component includes lithogenic and biogenic metals (i.e., Ca, Sr, Fe and Mn) and some anthropogenic metals (i.e., Ni, Co, Cu and Zn). The second component includes Hg and Pb, also considered anthropogenic metals. Our results show that the 1890s and 1900s were characterized by high concentrations of Ca and Sr, indicating the presence of carbonate algal communities in the harbour waters and suggesting absence of strong anthropogenic influence. The 1910s, 1920s and 1930s display a strong influence by Hg and Pb, most probably associated to the resurgence of the jewellery industry and the introduction of leaded gasoline in the early twentieth century.

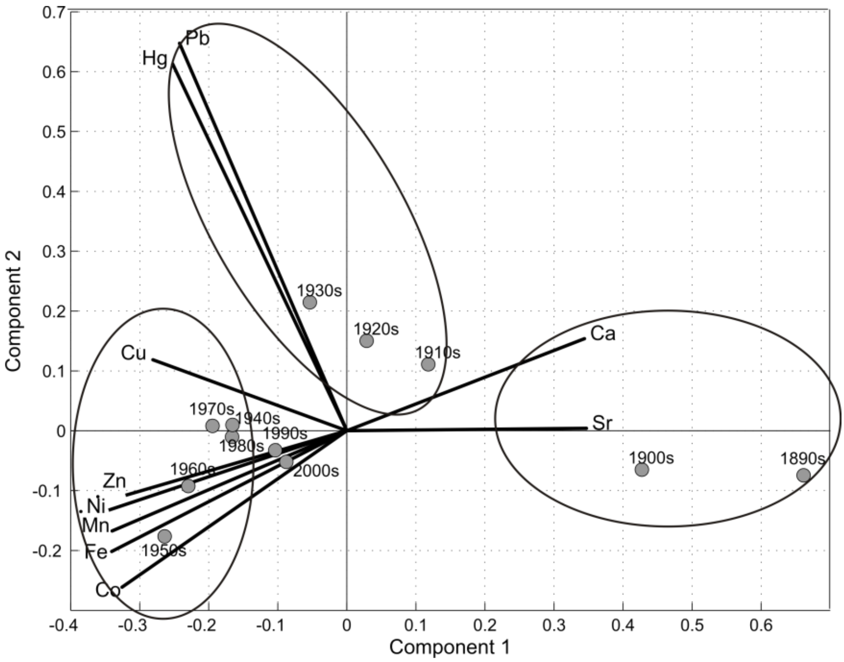

Figure 4.4 . Principal component Analysis (PCA) biplot for the different elements considered in this study. Grey dots and lines represent decades and averages by decades for trace metals, respectively.

4.3.3. Anthropogenic contribution and degree of contamination

The results of EF, Igeo and AL for the different elements of surface sediments in Mahon harbour are shown in Table 4.2. The values indicate that Fe, Mn, Co and Ni can be considered as minor enrichment, uncontaminated and under AL1 for the CEDEX normative. Aluminum-normalized Hg, Pb, Cu and Zn values are higher than continental crustal values (Wedepohl, 1995). Copper and Zn EF values correspond to moderately severe enrichment whereas that for Pb and Hg indicate a very severe and extremely severe enrichments, repectivley. Results from the I_{geo} index indicate that Cu and Zn are uncontamined to moderately contamined, Pb is moderately to strongly contamined, and Hg is strongly to extremely contaminated. According to the CEDEX normative, Cu and Zn concentrations fall below the AL1 and Hg and Pb are between AL1 and AL2. Our results indicate that Pb, Cu, Hg and Zn concentrations in sediments are probably the result of anthropogenic activity, with concentrations that suggest an important pollution.

Table 4.2 Enrichment factor (EF), geoaccumulation index (Igeo) and actions limits level (CEDEX) for metal concentrations in surface sediments of obtained in this study. (ver anexos p. 194)

	Average	SD	EF	Igeo	Actions Limits (CEDEX)
Fe	19313.45	0.50	1.76 Minor enrichment	-1.75 Uncontaminated	NA
Mn	201.15	47.22	1.10 Minor enrichment	-2.42 Uncontaminated	NA
Ni	18.10	5.00	1.27 Minor enrichment	-2.21 Uncontaminated	AL1
Co	5.33	1.24	0.87 No enrichment	-2.76 Uncontaminated	NA
Pb	97.65	25.03	25.94 Very severe enrichment	2.14 Moderately to strongly contaminated	AL1 - AL2
Zn	113.48	26.59	6.86 Moderately severe enrichment	0.22 Uncontaminated to moderately contaminated	AL1
Cu	64.67	17.55	10.17 Moderately severe enrichment	0.79 Uncontaminated to moderately contaminated	AL1
Hg	1.01	0.29	98.81 Extremely severe enrichment	4.07 Strongly to extremely contaminated	AL1 - AL2

Calculation of the EF, I$_{geo}$ and AL values for Hg and Pb in our core sediments (Figure 4.5) clearly showed that the concentrations of metals in the sediments are linked to the industrial history of the Mahon Harbour.

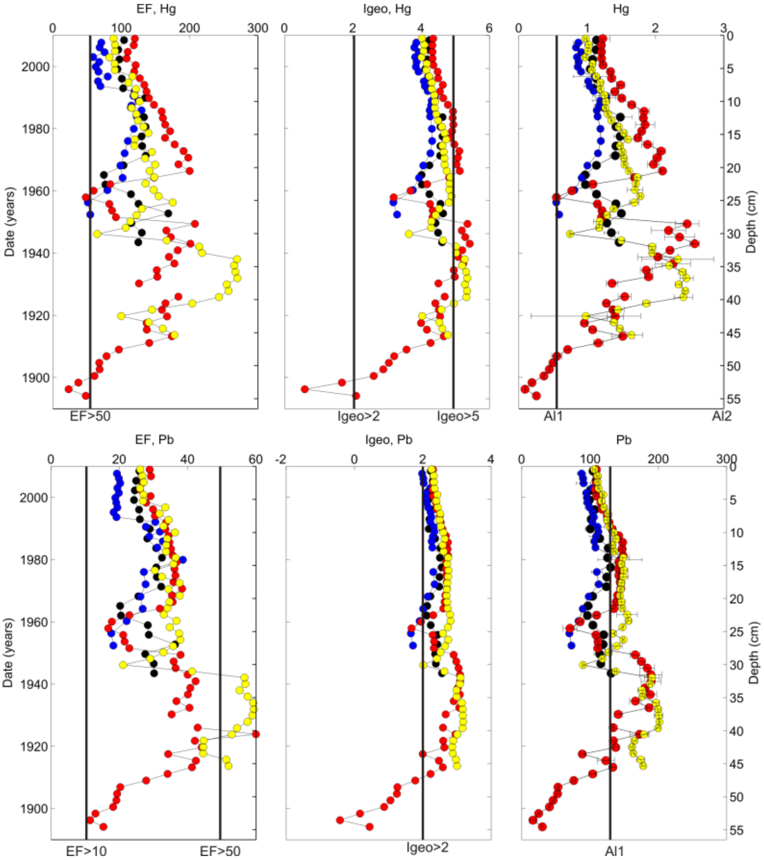

Figure 4.5 Profiles of enrichment factors (EF), Igeo and AL for Hg and Pb for each of the four cores collected in Mahon Harbour. Continuous lines represent the different factor levels.

At the end of the XIX century, the EF's of Hg and Pb were above the extremely severe and severe enrichment classifications, respectively, coinciding with growth of the marine, motor, textile, jewellery and shoe industries. The I_{geo} index show values that are above of the strongly contaminated classification for Hg, and above the moderately contamined for Pb. Regarding the action limits of the CEDEX normative, the Hg values are mostly between AL1 and AL2, whereas those of Pb are around AL1. Our results can be especially relevant for the port authorities since it could help them to evaluate contamination risks during dredging operations and harbour works.

4.3.4. Assessment potential pollution (Degree of Pyritation (DOP), Ni/Co ratio and Corg)

The total amount of pyrite formed in a marine environment is strongly dependent on its redox characteristics (e.g., oxic, anoxic-nonsulfidic, anoxic-sulfidic; Berner, 1984). Huerta-Diaz and Morse, (1992) found that sedimentary pyrite is an important sink for Hg, moderately important for Co, Cu, Mn, and Ni, and generally unimportant for Pb and Zn, all of which are of environmental, biological, and geochemical importance. Several authors indicate that Ni and Co are stable enough to become associated with pyrite (Algeo and Maynard, 2004; Huerta-Diaz and Morse, 1992). In reduced sediment environments and in the presence of sulphide, both DOP and Ni/Co ratio has been used as a redox index in different sedimentary environments (Dypvik, 1984; Jones and Manning, 1994; Rimmer et al., 2004; Śliwiński et al., 2010). Also, relationships between C_{org} and Ni/Co ratios may provide clues about sedimentary redox conditions of organic matter (Rimmer et al., 2004). This is why DOP, Ni/Co ratio and Corg content can be used as indicators of the potential pollution.

Profiles of DOP versus depth showed a particular trend (Figure 4.6), all presented the higher values (~ 50%) from the bottom to the 15 cm (~ 1980s) and then gradually decreased to the top. Jones and Manning, (1994) suggest that sediment samples from dysoxic environments have DOP values falling within the range 46-80%, accordingly, almost all sediment cores are under a very low oxygen concentration. The Ni/Co ratio in Mahon core sediment has values lower than 5 (Figure 4.6), which could imply oxic conditions in sediments according to Jones and Manning, (1994). Organic carbon contents in core sediments of Mahon harbour do not have a particular trend (Figure 4.6) and all values are lower than 5%, which implies low organic matter content. In this case the DOP, Ni/Co and Corg results do not agree. But, it can be assumed that the Ni/Co ratio could be lower than proposed, by the fact that sediments of Mahon harbour have low contents of Corg and have gone through different deposition conditions. Rimmer et al., (2004), found Ni/Co ratios indicating dysoxic conditions in sedimentary rocks with more or less 5% of organic carbon content and concludes that lack of a good correlation between parameters likely reflects different processes or conditions responsible for accumulation of such elements. Which leads to affirm that sediment cores of Mahon Harbour are in dysoxic conditions and any significant physical disturbance of the sedimentary environment (e.g., dredging or strong storm) can potentially oxidize the pyrite phase placing significant amounts of some trace metals (e.g. Hg and Pb) into solution in the water column (Carvajal and Landergren, 1969; Huerta-Diaz and Morse, 1992).

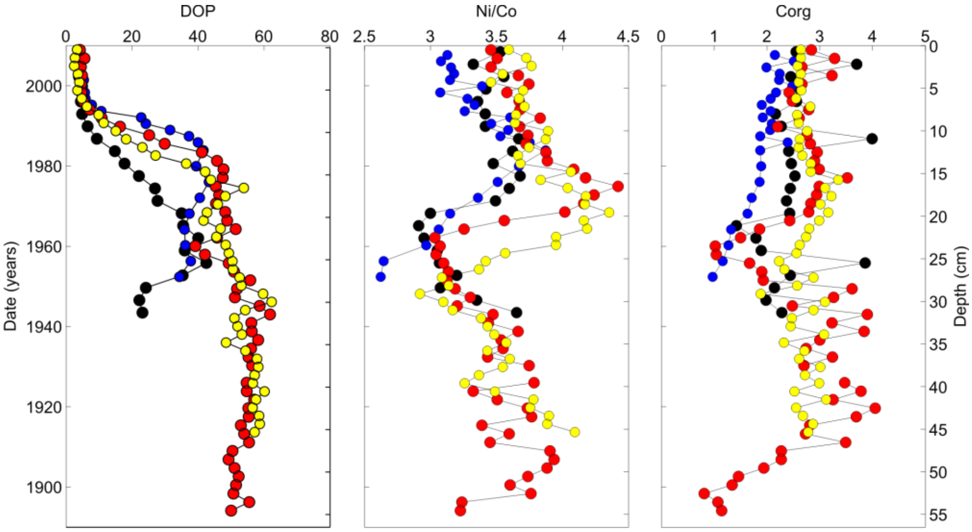

Figure 4.6 Profiles of DOP, Ni/Co and Corg, for each of the four cores collected in Mahon Harbour.

4.4. Conclusions

The distribution of trace metals in marine surface sediment of Mahon harbour reveals a clear difference between lithogenic (Fe, Mn, Ni and Co) and anthropogenic (Hg, Pb, Cu and Zn) metals. In general, all studied trace metals haves an affinity with fine grain size, but in the inner harbour it can be noted that it could also respond to a local source, especially Hg, Pb, Cu and Zn. Compared with other Mediterranean coastal areas, trace metal concentrations obtained in this study are higher or similar, suggesting that Mahon harbour is a coastal zone with particular anthropogenic pressure. Mahon harbour has been subjected to a progressive sediment contamination by metals due to the different types of industry established around the harbour in the last 120 years. Surficial metal concentrations of Hg, Pb, Cu and Zn are lower than old sediment record, but are still are higher than the recommended concentrations for acceptable sediment quality. The impact of anthropogenic metal pollution of Mahon harbour sediments was evaluated using metal enrichment factors (EF) geoaccumulation index (Igeo) and action limits (CEDEX) and confirm anthropogenic origin and metal pollution in the Mahon harbour, particularly with regard to Hg and Pb. Likewise, the degree of pyritization (DOP) and the Ni/Co ratio confirm the dysoxic conditions in sediment profiles, leaving the polluting potential evidence that may have these sediments if exposed to the surface.

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Discusión General

Los ecosistemas costeros constituyen un componente fundamental del medio marino. Estos sistemas reciben y procesan gran parte de los aportes procedentes de las descargas fluviales, la deposición atmosférica, las descargas de aguas residuales y/o la descarga de aguas subterráneas y en algunos casos de los intercambios con alta mar (Jickells, 1998). Los nutrientes circulan entre los componentes del ecosistema (bióticos y/o abióticos), sufriendo transformaciones que están reguladas por distintos procesos biogeoquímicos. El tiempo que cada elemento permanece en un compartimento se denomina tiempo de residencia (Spellman, 2008). La renovación del agua costera está regulada por factores como la geomorfología de la costa, la estratificación vertical de la columna de agua, las corrientes oceánicas y los forzamientos meteorológicos, entre otros. La disminución en la renovación del agua puede favorecer la potencial acumulación de nutrientes y otros elementos aportados por las distintas fuentes costeras (ríos, aguas subterráneas, vertidos, etc.)

Este trabajo analiza los procesos y transformaciones biogeoquímicas que afectan al funcionamiento ecológico de un ecosistema costero mediterráneo con intercambio restringido, el Puerto de Mahón. Nos hemos centrado en el análisis de los dos principales compartimentos ambientales: los sedimentos y la columna de agua. Para cumplir con este objetivo el trabajo se organizó a lo largo de cuatro líneas principales de investigación. La primera, analiza la dinámica de las aguas del puerto, su estacionalidad y respuesta a forzamientos de episodios atmosféricos. La segunda, caracteriza la composición química de las aguas y sedimentos superficiales de Mahón y evalúa las posibles fuentes de nutrientes y metales traza. La tercera, evalúa la respuesta del fitoplancton marino a los distintos escenarios biogeoquímicos que ocurren estacionalmente en el ecosistema de Mahón. Finalmente, la cuarta línea identifica y describe los cambios históricos que ha sufrido el funcionamiento biogeoquímico de Mahón como consecuencia de las variaciones naturales y antropogénicas ocurridas en el último siglo.

El análisis de un año de datos meteorológicos, hidrográficos, y físico-químicos revela que, el estado ecológico del ecosistema marino de Mahón y los procesos biogeoquímicos que lo determinan están dominados por una fuerte estacionalidad hidrodinámica. La hidrodinámica del sistema está determinada por el equilibrio entre los aportes internos y los intercambios con las aguas exteriores al estuario. En ausencia de un forzamiento dominante, el comportamiento del estuario obedece a forzarmientos que, asimismo, presentan una fuerte estacionalidad. Según Largier, (2010), la mayoría de los estuarios en clima mediterráneo con intercambio restringido tienen una marcada variabilidad estacional, alternando entre un estado clásico en la estación húmeda y un estado inverso en la estación seca. En Mahón, durante la estación húmeda los aportes por escorrentía, producto de las lluvias típicas de esta temporada, inducen una circulación estuarina con un transporte neto hacia el exterior en superficie y circulación hacia el interior en las capas profundas. Es importante entender que este esquema conceptual, identificable por un gradiente negativo de salinidad hacia el interior del estuario, no es generalmente estable, sino que está constituido por una serie de movimientos oscilatorios que modulan el flujo. En verano, la práctica ausencia de aportes de agua dulce, y la elevada evaporación y la estratificación térmica de las aguas son las características más relevantes. La renovación de las aguas queda a expensas de un régimen de vientos más débil y pueden ocurrir fenómenos de estancamiento de las aguas que se extienden durante varios días. El equilibrio en estos estuarios mediterráneos de poco flujo y marcada estacionalidad puede ser perturbado brevemente por eventos episódicos como lluvias torrenciales y/o tormentas (Largier, 2010). En Mahón ocurren importantes eventos de lluvias torrenciales y tormentas entre otoño y primavera asociados al paso de depresiones atmosféricas, esto produce un importante aporte de agua dulce que se ve reflejado en una buena correlación con la disminución de la salinidad de las aguas del ecosistema. Esta disminución solo se limitó a las aguas superficiales durante escaso tiempo (5 ± 2 días), lo que demuestra una rápida recuperación y que el estuario es más resistente a las perturbaciones episódicas de lo esperado.

La importancia de la dinámica de los vientos locales en los flujos y la renovación de agua en este tipo de ecosistemas ha sido demostrada por varios autores (e.g. Geyer, 1997; Hearn and Robson, 2002; Llebot et al., 2014). Sin embargo, hay que señalar que, debido a la elevada profundidad de la columna de agua y a su fuerte estratificación, este flujo en el periodo de verano se limita a la capa superficial dejando la capa profunda casi en condición de estancamiento. Además, existe una gran polarización en el forzamiento eólico con peridos de largas calmas y episodios de Tramontana. En consecuencia, aunque los flujos de aguas diarios entre las dos temporadas sean comparables (9,4 ± 5 y 8,1 ± 5), en verano la renovación del agua en el puerto de Mahón es más episódica y afecta casi exclusivamente a la capa superficial. A partir de medidas isotópicas, Rodellas et al., (2015) estimaron que Mahón tarda una media de 6,7 ± 1,8 días en renovarse durante el verano.

La estacionalidad en los flujos terrestres conlleva una variación estacional en los aportes de nutrientes inorgánicos. Las concentraciones de la mayoría de los nutrientes inorgánicos (DIN, P y Si) aumenta en la temporada de lluvias (de otoño a primavera) coincidiendo con la disminución de la salinidad superficial, lo que indica su estrecha relación con los aportes terrestres asociados a las lluvias. Esta estacionalidad asociada con descargas fluviales ya es conocida en los sistemas costeros mediterráneos (e.g. Ramírez et al., 2005; Sebastiá et al., 2013; Tuncel et al., 2007; Zaldívar et al., 2003). A pesar de las variaciones estacionales, la concentración de los nutrientes inorgánicos mantiene un patrón relativamente similar durante todo el año, mostrando un gradiente desde el interior del estuario hacia el exterior. Este gradiente refleja la importancia de los aportes terrestres (naturales o antrópicos) en la cabecera del estuario. Además de los aportes por escorrentía, reflejados principalmente en las variaciones de DIN y Si, el aumento de la concentración de NH_4^+en la época de verano, sugiere que existe un reciclado interno importante y/o un aporte de origen antropogénico. Aunque no se encontró una buena correlación estadística entre la disminución de salinidad y el aumento de la concentración de nutrientes durante los eventos episódicos de lluvia, la respuesta inmediata de la salinidad ante estos eventos deja en evidencia la importancia de los aportes terrestres y la influencia que estos pueden llegar a tener en los procesos biogeoquímicos del ecosistema, aunque sean de corto alcance temporal. Todo esto señala que existen dos importantes fuentes de nutrientes que se superponen: una fuente asociada a aportes antropogénicos y otra asociada a los eventos episódicos. Las variaciones en estas fuentes, junto con los procesos biogeoquímicos internos, determinan el balance de nutrientes del estuario.

Como resultado de todos esos aportes Mahón se encuentra enriquecido en DIN, principalmente en la parte interna del estuario. Este hecho se refleja en las relaciones molares N:P, que son superiores a la relación de Redfield durante casi todo el año . Esta relación muestra una disminución hacia la parte externa del puerto en donde se alcanzan valores de 16 o inferiores. Asimismo, se observan pequeñas variaciones locales en esa relación molar N:P, que también se pueden asociar a las mismas variaciones observadas para las concentraciones del DIN. Estas variaciones en la relación N:P, asociadas a las variaciones de DIN, tienen como consecuencia la limitación por P en diferentes grados. Hemos analizado esta limitación mediante el uso de APA, tal como discutimos más adelante.

Como consecuencia de las variaciones en las relaciones molares N:P, la producción primaria en el ecosistema varía, reflejado en las variaciones espacio-temporales de la Chl *a*. En general, la Chl *a* presenta una marcada variación temporal, típica de este tipo de ecosistemas costeros eutrofizados, mostrando sus máximos a finales de verano y principios de otoño. También se pueden observar variaciones en la parte interna del puerto, donde la biomasa parece responder a procesos más locales. Durante casi todo el año las concentraciones de Chl *a* en la parte interior del puerto son elevadas (up 5.96 $mg{\cdot}m^{-3}$), sin embargo, existe un factor de dilución durante la temporada de lluvias y sobre todo con posterioridad a eventos fuertes de escorrentía. Esto sugiere que los aportes de DIN de las lluvias muy intensas podrían estar limitando la producción, probablemente debido a los cambios producidos en la relación molar N:P. Sin embargo, es sabido que esta relación en sí misma no justifica la limitación de nutrientes, varios autores han indicado que los valores altos o bajos de la relación molar N:P no indican necesariamente la limitación del fitoplancton o deficiencia de fosfato (Geider and Roche, 2002; Guildford and Hecky, 2000; Rees et al., 2009; Thingstad et al., 1998).

Un factor importante para entender los procesos que controlan la producción primaria y sus limitaciones, es conocer el tipo y variaciones del fitoplancton que co-existen en el estuario. En el caso de Mahón, el fitoplancton responde a las marcadas variaciones temporales, siendo los dinoflagelados y los cocolitoforidos los taxones que prevalecen durante todo el año, con su mayor abundancia en primavera y verano cuando las condiciones de nutrientes, temperatura, salinidad y baja hidrodinámica favorece la presencia de estos. Al comienzo del otoño disminuye la abundancia de los dinoflagelados y los cocolitoforidos y aparecen las diatomeas, llegando a dominar hasta un 80% de la abundancia del fitoplancton, pero esta dominancia dura poco tiempo, lo que se atribuye a la baja disponibilidad de Si. Esto muestra una sucesión fitoplanctónica particular en el estuario, dominada por la presencia de especies motiles durante todo el año. Además, el estudio de la actividad de la fosfatasa alcalina muestra resultados interesantes en cuanto a la limitación de nutrientes y la dinámica del fitoplancton en Mahón. Durante las dos temporadas evaluadas el fitoplancton exhibe actividad de fosfatasa alcalina, sugiriendo estrés en el fitoplancton por poca disponibilidad de fosfato. Este hecho pone en evidencia la particularidad de los procesos biogeoquímicos de este ecosistema. Si bien las concentraciones de fosfato en el estuario (0,09 ± 0,02 y 0,06 ± 0,02 μmol l^{-1} en junio y octubre de 2011 respectivamente) son similares y hasta menores a otras zonas costeras del mediterráneo (e.g. Basterretxea et al., 2010; Diaz et al., 2001; Moncheva et al., 2001; Rodellas et al., 2014; Tovar-Sánchez et al., 2016, 2014). En general, las concentraciones de fosfato son ligeramente superiores a la media de fosfato estimada en el Mediterráneo (~0,04 μmol l^{-1}) y esta condición parece perpetuarse durante todo el año, producto de los aportes terrígenos que recibe. Los resultados del bioensayo realizado en el presente trabajo muestran actividad de la fosfatasa alcalina aun cuando concentraciones de fosfato añadidas son altas. Ello sugiere que existe un uso preferencial del fosfato orgánico por parte de algunas especies de fitoplancton. Para ambos casos, tanto las campañas evaluadas como el bioensayo, los dinoflagelados son el taxón responsable de la mayoría de la actividad de la fosfatasa alcalina observada, coincidiendo con lo observado en otros estudios (e.g. Dyhrman and Ruttenberg, 2006; Ou et al., 2006; Rengefors et al., 2003). El hecho de que haya actividad de la fosfatasa alcalina, incluso cuando hay mayor disponibilidad de nutrientes, señala la diversidad de estrategias de supervivencias ya conocida en los dinoflagelados. Así, algunos dinoflagelados compensan su baja eficacia al competir por recursos, obteniendo el fosfato necesario desde diferentes fuentes, como por ejemplo el DOP (Dyhrman and Palenik, 1999; Dyhrman and Ruttenberg, 2006; Nicholson et al., 2006). El resultado del bioensayo también sugiere que algunos dinoflagelados pueden tener un tiempo de respuesta y adaptación de su fisiología más lento ante la mayor disponibilidad de fosfato en el medio. Esto evidencia que la actividad de la fosfatasa alcalina *per se* en especies del fitoplancton, al igual que la relación molar N:P, no necesariamente es un indicador de estrés y/o limitación por fosfato y que ambos indicadores son el resultado tanto de las condiciones ambientales, como de las condiciones fisiológicas de las especies presentes en el medio (Cao et al., 2009; Mackey et al., 2007; Meseck et al., 2009; Nicholson et al., 2006).

Si bien se sabe que, las concentraciones de nutrientes pueden tener un control de primer orden sobre la producción primaria en el medio marino, otros factores tales como co-limitación por metales traza, también tiene una influencia importante, sobre todo en cuerpos de agua semi-cerrados que reciben importantes aportes de origen antropogénico, como el caso de Mahón. De aquí deriva la importancia de evaluar el contenido y la fuente de metales traza en este tipo de ecosistemas. Los resultados muestran que, al igual que los nutrientes, la mayoría de los metales exhibe un gradiente de concentración que disminuye hacia la boca del estuario. Esto señala, una vez más, la importancia de los aportes (naturales o no) que existen en la parte más interna del puerto y el intercambio que este tiene con las aguas exteriores. Al igual que ocurre con los nutrientes, metales como Co, Cu, Ni, Pb y Zn, también muestran variaciones espacio-temporales que sugieren fuentes asociadas a actividades antropogénicas que pueden descargar directamente al ecosistema o provocadas por eventos naturales como los episodios de lluvias. Por su parte, el hecho de que las concentraciones promedio de la mayoría de metales en las aguas superficiales de Mahón (sobre todo para Cu 21 ± 8 nM, Fe 9 ± 3 nM y Pb 1,3 ± 0,4 nM), sean superiores a las reportadas para otros ecosistemas costeros del mediterráneo, hace sospechar la existencia de una fuente continua de estos elementos al medio. Garcia-Orellana et al., (2011) señalaron que, debido a los diversos tipos de industria establecidos alrededor del puerto ha ocurridos una progresiva contaminación de los sedimentos por metales (por ejemplo, Pb y Cu) y que estos podrían ser una de las fuentes que causa estas altas concentraciones medidas en sus aguas. No obstante, existen diversas fuentes para los metales en estos ecosistemas y sus variaciones están sujetas a las condiciones ambientales y químicas del mismo.

En el caso de Mahón, se han evaluado las fuentes de metales que se consideran de mayor importancia. La escorrentía en la cuenca del estuario es efímera ymuy reducida durante todo el año. Sin embargo, al evaluar las condiciones ambientales del estuario se observa que éste recibe aportes episódicos de agua dulce que pueden ser torrenciales durante el otoño. El torrente de Sant Joan, aparte de ser la fuente más importante de agua dulce, tiene concentraciones de metales traza más elevadas que las observadas en las aguas del estuario (Co: 4,4 ± 3,2 nM, Cd: 0,2 ± 0,2, Cu: 53,9 ± 7,1 nM, Fe: 677 ± 375 nM, Mo: 37,5 ± 29,5 nM, Ni: 62,4 ± 2,1 nM, Pb: 1,9 ± 0,2 nM, y Zn: 70,9 ± 8,5 nM), pudiendo ser parcialmente responsable de las altas concentraciones de algunos metales traza observadas en la parte más interior del mismo. La descarga submarina de aguas subterráneas también es considerada una fuente importante de metales en zonas costeras (Beck et al., 2007; Rodellas et al., 2014; Tovar-Sánchez et al., 2014; Trezzi et al., 2016; Windom et al., 2006). En Mahón las concentraciones de metales medidas en los pozos son importantes para algunos metales, pero hay que considerar que la descarga directa de agua dulce puede no ser la principal fuente de estos al ecosistema, ya puede existir una importante recirculación de aguas de mar a través de los sedimentos, como se ha indicado para otras zonas del Mediterráneo (Rodellas et al., 2015a). La deposición atmosférica podría ser otra fuente importante de metales en este tipo de ecosistemas. Se ha demostrado en otros estudios como las deposiciones atmosféricas pueden ser un gran aporte de metales al medio marino (e.g. (Guerzoni et al., 1999; Guieu et al., 1997; Jordi et al., 2012). En el caso de Mahón su aporte se calcula a partir de datos de flujos ya existentes para el mediterráneo (Guieu et al., 1997) tomando en cuenta el área superficial del cuerpo de agua donde tendrían influencia las deposiciones. Por su parte, las concentraciones de metales como Cu, Hg, Pb y Zn en los sedimentos superficiales son mayores en comparación a otros lugares costeros del Mediterráneo (entre 28 - 75%, 30 - 98%, 42 - 95% y 12 - 48%, respectivamente), coincidiendo con las altas concentraciones halladas en las aguas superficiales para Cu, Pb y Zn. Existen diversos estudios que confirman el origen antropogénico de estos metales, señalando como se acumulan y enriquecen en los sedimentos y el impacto que estos tienen en los ecosistemas costeros (Aloupi and Angelidis, 2001; Buccolieri et al., 2006; El-Sorogy and Attiah, 2015; Nemr et al., 2006; Pekey, 2006; Zhang and Liu, 2002).

La evaluación de los flujos de metales, mediante un balance de masas, permite discernir qué fuente es la que realiza los aportes de mayor importancia en Mahón. Una vez calculados los flujos de exportación hacia mar abierto, descarga de aguas subterráneas, deposición atmosférica y escorrentías, el flujo proveniente de los sedimentos resulta ser la fuente más importante para los metales estudiados (i.e. Co, Cu, Fe, Ni, Pb y Zn). Se estima que este flujo supone entre un 80 a un 99% de los aportes al ecosistema. Si bien, son varios los procesos que son responsables de los flujos de metal desde los sedimentos hacia la columna de agua, en Mahón la resuspensión de sedimentos representa el mecanismo más importante. Esta resuspensión es provocada por el tráfico marítimo y se considera uno de los factores más importantes debido a la frecuencia y magnitud con la que ocurren estos eventos en el puerto. Mahón, debido a su gran importancia mercantil y turística recibe embarcaciones de gran calado con mucha frecuencia, sobretodo en la época estival. Si a esto se le suma la poca profundidad de la parte interior del puerto, se dan las condiciones propicias para la resuspensión de sedimentos y la transferencia de contaminantes hacia la columna de agua (Garcia-Orellana et al., 2011; Rodellas et al., 2015b). Esto se sustenta con las buenas correlaciones entre las concentraciones de los metales y los isoptopos de Ra (^{223}Ra y ^{224}Ra) en las aguas superficiales del estuario que, según Rodellas et al., (2015b), provienen de la desintegración de sus isotopos padres (^{227}Ra y ^{228}Ra) que se hallan en el sedimento.

Factores como, las diversas actividades portuarias, el tráfico marítimo y la poca profundidad en la parte interna del estuario, tienen gran impacto en su fondo marino. Estos factores, en concomitancia con el contenido de metales en las aguas, señalan a los sedimentos como una de las fuentes potenciales de estos metales en el estuario, tal como muestra el balance de masas realizado. Los núcleos de sedimentos datados en el presente estudio, proporcionan información de las variaciones en los metales desde el año 1890 hasta el presente. Estos datos muestran que el contenido de metales en el sedimento de Mahón es el resultado de las diversas actividades desarrolladas históricamente en esta zona. Así, se observa un marcado aumento en las concentraciones de Hg y Pb entre los años 1910, 1920 y 1930, probablemente asociado al auge de la industria textil, joyera y la utilización de la gasolina con plomo. Por otro lado, durante la segunda guerra mundial y el periodo postguerra (1945-1947), se observan variaciones en la concentración de la mayoría de los metales, reflejando así el cese y lenta reactivación de las actividades industriales. Esta tendencia se mantiene hasta los años 50 en los que se liberalizaron las políticas económicas. Producto de la reactivación e implantación de nuevos sectores industriales (textil, joyera, zapatería, electrónica, lácteos, la implantación de centrales termoeléctricas, entre otras) se originaron nuevos aportes de metales como Hg, Pb Cu y Zn al sedimento, provocando el incremento de sus concentraciones entre las décadas de los 50 y 70. En 1978, la construcción de un emisario con salida al mar, que evacua los residuos de las fábricas y residuos urbanos fuera del puerto, permitió la reducción de la concentración de metales en las aguas del puerto, lo que se traduce en una disminución continua y la estabilización del contenido de Hg, Pb, Cu y Zn en sedimentos a partir de la década de 1980 hasta ahora. Por su parte, los resultados del cálculo del factor de enriquecimiento (EF) y del índice de geoacumulación (Igeo) muestra que los sedimentos de Mahón están enriquecidos en Cu, Hg, Pb y Zn, considerándose en algunos casos contaminados. Estos resultados implican que cualquier alteración física significativa del ambiente sedimentario (por ejemplo, el dragado o fuerte tormenta) puede provocar la resuspensión de cantidades significativas de algunos metales traza (p. ej. Hg y Pb) y su consiguiente disolución en la columna de agua (Carvajal and Landergren, 1969; Huerta-Diaz and Morse, 1992).

La presente tesis doctoral ha contribuido a mejorar el conocimiento sobre los procesos biogeoquímicos que rigen un ecosistema costero con intercambios restringidos en ambiente mediterráneo, el puerto de Mahón. Se ha demostrado que, aunque existe una marcada estacionalidad en el estuario, otros factores como los eventos episódicos de lluvias y los forzamientos por vientos, son de importancia en los procesos biogeoquímicos entre nutrientes, metales y producción primaria. También, se ha demostrados que existen diversas fuentes de metales y nutrientes que influyen en el estado geoquímico y fisiológico del estuario. Que las principales fuentes de entrada de metales y nutrientes a la columna de agua son los sedimentos enriquecidos y/o contaminados por la actividad industrial histórica en el puerto y las descargas puntales de escorrentías producto de lluvias torrenciales que transportan hacia el estuario los productos de las actividades antropogénicas que allí se desarrollan. Que estas fuentes tienen su mayor importancia e impacto en la parte interior del ecosistema, debido a su morfología, poca profundidad y mayor influencia antropogénica. Igualmente se ha evidenciado que el ecosistema parece tener una capacidad de recuperación bastante rápida a los impactos de los eventos episódicos y que tiene sus propias estrategias de adaptación para mantener sus condiciones biogeoquímicas.

Los resultados de esta tesis apuntan a distintas líneas de trabajo futuro en relación a mejorar los conocimientos sobre la importancia de las diferentes fuentes de nutrientes y metales en el estuario y su influencia a largo plazo en los procesos biogeoquímicos que rigen en él. En primer lugar, dada la importancia en los procesos biogeoquímicos de los aportes de nutrientes y metales por diferentes fuentes, el obtener series de datos de los flujos y concentración de nutrientes y metales provenientes de dichas fuentes (aguas subterráneas, escorrentías, deposición atmosférica, sedimentos) representaría una mejora significativa para cuestiones como por ejemplo determinar patrones de entradas de nutrientes y metales, entender los cambios que suceden a diferentes escalas temporales, las cuales pueden estar muy influenciadas por la variabilidad intrínseca de los factores, realizar balances de masas más detallados que permitan discernir la importancia de cada fuente para cada caso (nutriente y metales) y si hay variaciones a diferentes escalas temporales, y también se podría evaluar la dinámica mediante distintos tipos modelos. En segundo lugar, debido al interés de conocer de manera más detallada el estado fisiológico del fitoplancton presente en el estuario y su respuesta a los diferentes aportes de nutrientes y micro-nutrientes, se puede obtener una serie temporal de los diferentes taxones de fitoplancton que co-existen en el estuario y la evaluación de la actividad de la fosfatasa alcalina, junto al contenido de nutrientes de las aguas. Del mismo modo, se pueden desarrollar diferentes experimentos que permitan establecer la respuesta del fitoplancton ante diferentes escenarios de aportes. Por último, las implicaciones de los diferentes escenarios de forzamiento físico, eventos episódicos de lluvias y tiempos de residencia para las poblaciones de fitoplancton y los procesos biogeoquímicos que rigen el estuario, podrían ser exploradas con el acoplamiento de diferentes modelos (hidrodinámico, ecológico y químico). Modelos acoplados validados con datos de campo adecuados que podría proporcionar información sobre la dinámica del fitoplancton y los procesos biogeoquímicos y sus respuestas potenciales a diferentes escenarios climáticos.

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Conclusiones

El Puerto de Mahón es un ecosistema singular pero representativo de un estuario semicerrado en ambiente mediterráneo, un entorno natural sobre el que existe un conocimiento bastante limitado. La identificación y/o interpretación de los procesos biogeoquímicos y la predicción de las respuestas de este tipo de ecosistemas a las perturbaciones humanas o naturales, son tareas bastantes complejas. Por estas razones, los resultados de este trabajo son un importante aporte para ampliar el conocimiento sobre este tipo de estuarios en ambiente mediterráneos, específicamente el puerto de Mahón, y en función de los cuales se pueden escribir las siguientes conclusiones:

- El estuario de Mahón es un ecosistema semi-cerrado con un intercambio de agua reducido. Este hecho da lugar a una zonación longitudinal caracterizada por gradientes negativos de nutrientes y metales disueltos entre su zona interna, sometida a una elevada presión humana, y el mar abierto. En este sentido, las actividades humanas en el interior del estuario afectan en mayor o menor medida a todo el ecosistema.

- Nuestros resultados demuestran que la interacción entre la hidrografía y las fuentes de nutrientes juega un papel importante en la regulación de las comunidades de fitoplancton en el estuario de Mahón, el cual podría, a largo plazo alterar toda la trofodinámica del plancton.

- Como en muchas otras áreas costeras del Mediterráneo, los patrones canónicos de estacionalidad del fitoplancton se han desplazado desde la proliferación típica de finales de invierno, a una biomasa máxima en verano que está sustentada por los aportes antrópicos. La elevada temperatura, la estratificación y el incremento de actividad biológica son típicas de esta estación en la que el funcionamiento de este ecosistema costero está particularmente desacoplado del ecositema de aguas abiertas.

- El verano es el periodo de mayor vulnerabilidad del ecosistema debido a la renovación de agua que depende críticamente del forzamiento del viento y de episodios esporádicos de lluvia que en esta época del año son muy marginales. Alteraciones en la magnitud, frecuencia y duración de estos regímenes de perturbación en esta temporada podrían provocar cambios notables en el funcionamiento del estuario.

- En verano se producen respuestas bióticas mayores que en otras temporadas. Dado que el enriquecimiento antrópico altera las relaciones molares de los nutrientes en favor del nitrato muchas especies de fitoplancton se ven obligadas a suplir las fuentes de fosfato inorgánico por compuestos orgánicos. Esto produce una amplia variedad de estrategias específicas de las especies para satisfacer sus requerimientos nutricionales y ventajas nutricionales para los dinoflagelados que, según los ensayos de marcage de enzima por fluorenscencia (ELF por sus sigles en ingles) realizados, muestran una mayor capacidad para la utilización de estas fuentes de P orgánico.

- Pese a la importancia de la estacionalidad, aproximadamente, el 59% de la variabilidad fitoplanctónica pertenece a una señal no estacional, impulsada por procesos, biogeoquímicos y físicos de escala eventual, como el enriquecimiento de nutrientes o la variabilidad del tiempo de residencia. En todo caso, el ecosistema parece ser bastante resiliente a este tipo de episodios.

- La distribución de metales en los sedimentos superficiales del puerto de Mahón revela una clara diferencia entre aquellos metales de origen litogénicos (i.e. Fe, Mn, Ni y Co) y metales antropogénicos (Hg, Pb, Cu y Zn). En general, todos los metales traza estudiados tienen una afinidad con un grano fino, sin embargo, en la parte interna se puede notar que estas concentraciones podrían responder también a una fuente local, especialmente para los de origen antropogénico. Comparado con otras áreas costeras del Mediterráneo, las concentraciones de metales obtenidas en este estudio son mayores o similares, sugiriendo que Mahon es una zona costera con una presión antropogénica particularmente elevada.

- El estuario de Mahon ha sido sometido a una contaminación progresiva del sedimento por metales, debido a los diferentes tipos de industrias que se han establecido a lo largo de la bahía en los últimos 120 años. Las concentraciones superficiales de Hg, Pb, Cu y Zn son inferiores a aquellas encontradas en registros anteriores, no obstante, son superiores a las concentraciones recomendadas para una calidad aceptable del sedimento. El impacto de la contaminación antropogénica en los sedimentos de Mahon fue evaluado utilizando factores de enriquecimiento (EF) e índices de geoacumulación (Igeo), confirmando el origen antropogénico y la contaminación por metales en los sedimentos del ecosistema, particularmente con respecto a Hg y Pb.

- Los sedimentos representan hoy en día la mayor fuente de Co, Cu, Fe, Ni, Pb y Zn a la columna de agua. La principal vía de resuspensión de este sedimento es el tráfico marítimo de grandes buques. Como consecuencia de la contaminación por metales en el sedimento, y de su continua transferencia a la columna de agua, los niveles de Cu y Pb en el puerto de Mahon, son considerablemente mayores a los valores reportados en otras áreas mediterráneas.

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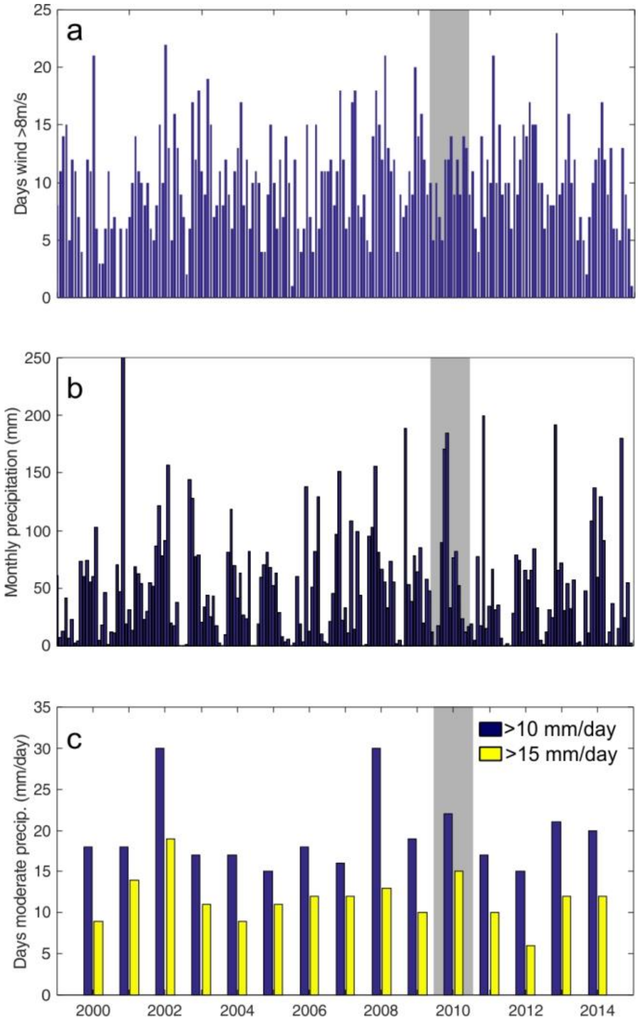

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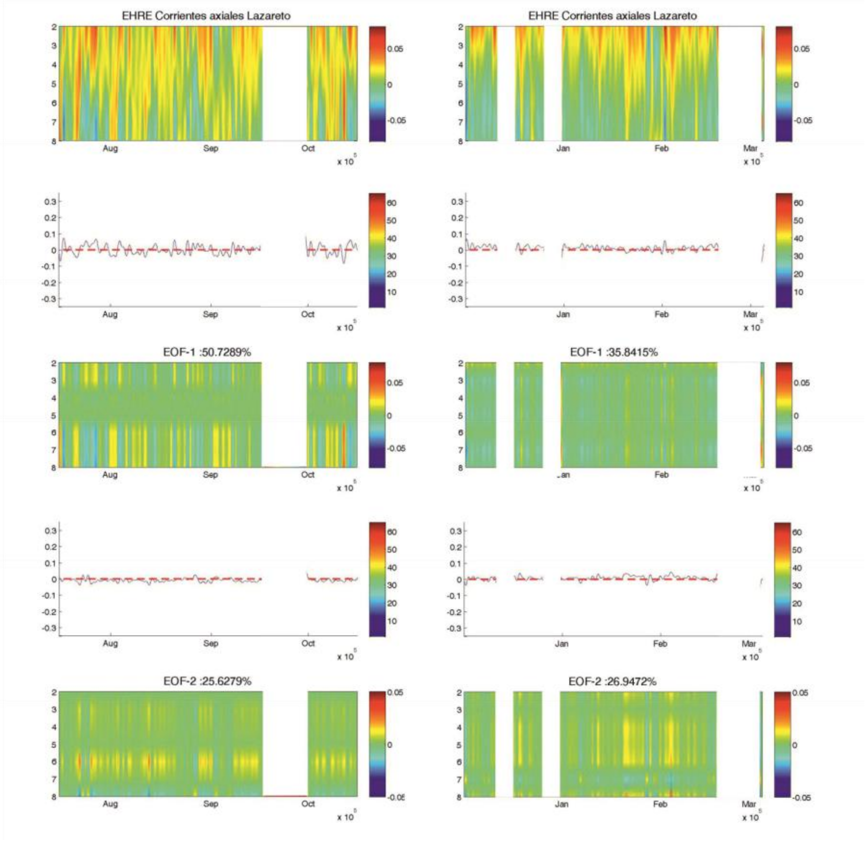

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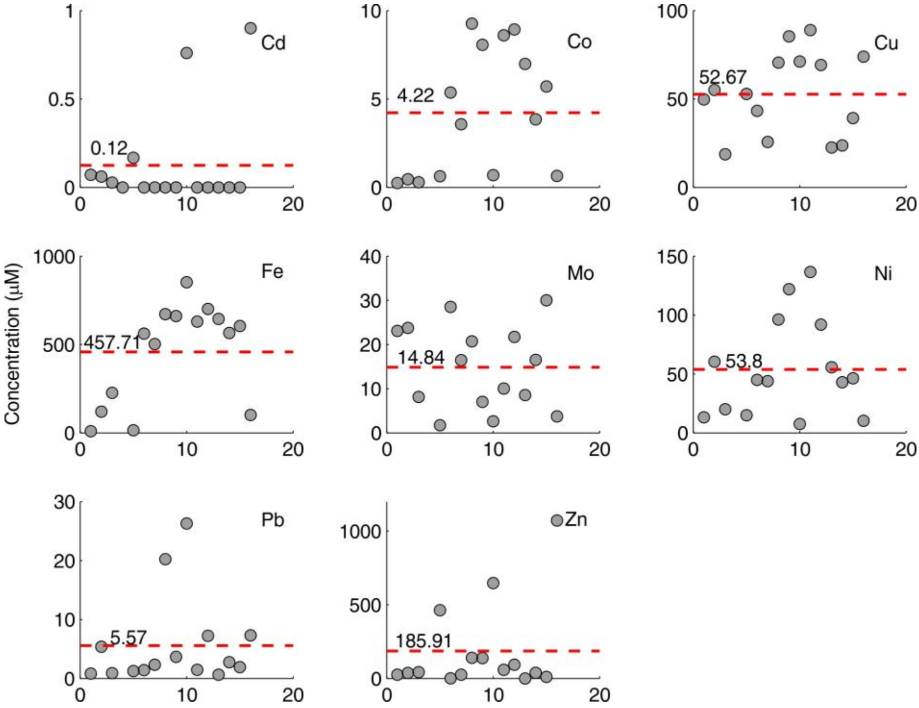

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Table S1. 1 Major tidal constituents at Mahon harbor. Percentage explained (PE), period (T), amplitude (A), phase (P).

Constituent	PE (%)	T (h)	A (cm)	A error	P (deg.)	P error
M2	49.68	12.42	5.02	0.0219	224.	0.241
K1	26.21	23.92	3.65	0.0356	173.	0.640
S2	8.17	12.00	2.04	0.0196	243.	0.542
O1	7.92	25.84	2.01	0.0403	106.	1.09
P1	3.31	24.04	1.30	0.0427	166.	1.60
N2	1.31	12.66	1.08	0.0177	210.	0.963
K2	0.66	11.96	0.579	0.0241	240.	2.15

Table S2.1 Median concentration of trace metals (nM) in seawater samples collected during four surveys in July 2010, October 2011, March 2010 and June 2011, repectively

Campaign	Station	Cd	Co	Cu	Fe	Mo	Ni	Pb	Zn
July 2010	**St1**	0.40	0.60	52.8	20.4	117	5.6	2.3	53.3
	St2	0.34	0.50	41.4	12.7	103	4.8	2.1	43.6
	St3	0.36	0.58	52.1	4.4	117	5.9	2.4	54.6
	St4	0.38	0.49	44.7	4.7	114	5.3	2.0	43.2
	St5	0.34	0.39	34.4	6.0	106	4.5	1.6	31.8
	St6	0.35	0.36	34.0	4.9	111	4.5	1.4	27.5
	St7	0.34	0.33	33.5	14.6	110	4.2	1.4	25.9
	St8	0.34	0.25	21.7	14.5	106	3.6	0.9	15.2
	St9	0.31	0.20	15.2	9.1	104	3.2	0.5	8.6
	St10	0.35	0.19	15.5	4.0	104	3.4	0.5	9.3
	St11	0.31	0.22	14.5	15.0	106	3.4	0.4	7.2
	St12	0.31	0.15	7.3	11.5	106	2.9	0.3	2.8
	St13	0.32	0.16	7.5	3.1	108	3.1	0.2	2.0
	St14	0.34	0.14	9.3	2.8	108	3.1	0.3	3.2
	St15	0.32	0.16	9.6	13.4	107	3.1	0.4	3.9
	Sream	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
October 2010	**St1**	0.34	0.49	29.0	34.7	109	5.4	1.6	41.8
	St2	0.35	0.43	17.3	15.2	112	4.8	1.6	25.0
	St3	0.34	0.49	20.3	11.1	116	5.2	1.7	27.7
	St4	0.36	0.44	18.7	10.4	123	5.2	1.6	23.8
	St5	0.36	0.42	18.0	8.5	119	4.8	1.6	22.7
	St6	0.35	0.36	15.6	7.6	121	4.6	1.4	17.7
	St7	0.36	0.35	13.9	7.9	119	4.5	1.2	15.3
	St8	0.33	0.32	12.4	7.9	119	4.4	1.2	13.7
	St9	0.34	0.28	9.7	7.1	114	4.0	0.9	9.5
	St10	0.34	0.27	9.8	10.5	116	4.0	2.4	18.0
	St11	0.37	0.26	8.2	7.6	117	4.1	2.3	12.7
	St12	0.33	0.25	8.6	6.9	116	4.0	2.2	10.8
	St13	0.35	0.23	8.8	6.4	115	4.1	1.9	9.2
	St14	0.38	0.20	6.2	5.1	122	4.0	1.9	7.7
	St15	0.34	0.18	4.0	5.2	120	3.6	1.4	4.9
	Stream	<LOD	7.62	60.9	1051.6	8	64.6	1.8	62.4
March 2011	**St1**	0.41	0.33	16.4	17.2	145	5.4	1.9	29.9
	St2	0.40	0.32	15.8	17.1	134	5.1	1.8	27.4
	St3	0.39	0.32	16.2	17.7	135	4.8	1.9	26.5
	St4	0.40	0.31	17.4	13.6	136	4.8	1.9	30.5
	St5	0.38	0.30	14.0	13.5	132	4.7	1.7	23.2
	St6	0.40	0.30	16.8	12.5	138	5.3	1.6	27.4
	St7	0.40	0.30	13.4	13.4	141	4.9	1.7	23.2
	St8	0.40	0.29	12.9	9.0	140	4.8	1.6	22.7
	St9	0.40	0.29	16.8	14.3	138	4.7	1.6	25.9
	St10	0.39	0.25	10.9	10.0	137	4.4	1.4	18.4
	St11	0.40	0.31	13.4	12.0	137	5.0	1.6	25.9
	St12	0.39	0.29	13.4	10.6	134	4.8	1.5	23.2
	St13	0.38	0.29	14.1	11.7	134	4.6	1.5	22.1
	St14	0.40	0.28	17.4	9.8	139	4.7	1.4	26.9
	St15	0.38	0.14	7.5	4.1	138	3.6	0.3	6.1
	Stream	0.31	1.20	46.8	302.4	67	60.3	2.1	79.4
June 2011	**St1**	0.17	0.35	35.4	8.8	114.1	4.2	1.2	42.9
	St2	0.20	0.36	36.9	10.0	121.4	4.5	1.4	40.5
	St3	0.18	0.41	53.6	14.4	118.8	4.7	1.3	58.8
	St4	0.22	0.37	42.7	11.0	116.3	4.4	1.2	45.3
	St5	0.17	0.30	33.9	4.1	116.0	4.2	0.9	35.7
	St6	0.20	0.31	43.0	4.2	121.2	4.3	1.0	45.1
	St7	0.16	0.30	37.4	2.9	113.0	3.8	0.9	37.9
	St8	0.18	0.27	36.0	2.0	120.3	4.0	0.8	34.5
	St9	0.18	0.22	24.7	2.6	114.8	3.8	0.6	23.6
	St10	0.16	0.22	22.3	1.7	117.5	3.8	0.6	21.5
	St11	0.18	0.20	18.2	2.6	114.3	3.5	0.5	17.9
	St12	0.14	0.18	13.0	3.2	112.5	3.3	0.4	15.5
	St13	0.18	0.18	12.4	1.8	113.1	3.4	0.3	13.4
	St14	0.17	0.16	11.6	1.2	116.9	3.3	0.3	14.4
	St15	0.12	0.12	5.6	1.6	95.4	2.7	0.2	6.3
	Stream	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.

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	Cd (nM)	Co (nM)	Cu (nM)	Fe (nM)	Mo (nM)	Ni (nM)	Pb (nM)	Zn (nM)
Ferrol Ria [a]	0.09 - 0.10		6.7 - 9.2				0.20 - 0.30	17 - 24
Vigo Ria [b]	0.02 - 0.10 (0.05 ± 0.02)	0.30 - 1.6 (0.67 ± 0.37)	1.1 - 5.3 (5.6 ± 2.4)			1.7 - 7.8 (4.1 ± 1.5)	0.08 - 0.25 (0.18± 0.07)	7.4 - 19 (13 ± 4)
Palma beach [c]			5.9 - 13	3.0 - 6.0	81 - 160	2.9 - 5.6	0.28 - 0.40	3.3 - 10
Cadiz bay [d]	(0.10 ± 0.03)		(6.7 ± 3.9)				(230 ± 150)	(17 ± 9)
Algeciras bay [d]	(0.2 ± 0.1)		(17 ± 8)				(14 ± 2)	(130 ± 60)
Majorca bays [e]	0.30 - 0.37	0.16 - 0.29	3.9 - 11	5.5 - 12	117 - 130	3.6 - 4.6	0.07 - 0.19	3.1 - 8.0
Majorca Shelf water [f]	0.34 - 0.37	0.14 - 0.17	4.5 - 9.3	2.4 - 3.6	120 - 133	3.7 - 4.2	0.10 - 0.13	2.0 - 6.6
Western Mediterranean [f]	0.06 - 0.08	0.05 - 0.17	1.1 - 2.3	1.6 - 8.0	107 - 140			
Eastern Mediterranean [f]	0.07 - 0.09	0.08 - 0.14	1.4 - 1.9	1.9 - 6.1	115 - 1230			
Aegean Sea [f]	0.08 - 0.12	0.12 - 0.29	1.8 - 4.4	2.1 - 4.0	110 - 130			
Marmara Sea [f]	0.10 - 0.11	0.45 - 0.52	7.7 - 8.6	3.7 - 4.9	55 - 61			
Black Sea [f]	0.06 - 0.09	0.18 - 0.37	6 - 8.8	1.8 - 3.7	39 - 47			
Maó Harbor [g]	0.1 - 0.4 (0.3 ± 0.1)	0.1 - 0.6 (0.30 ± 0.03)	2 -54 (21 ± 8)	1.2 - 35 (9.2 ± 3.2)	95 - 150 (119 ± 12)	2.7 - 5.9 (4.3 ± 0.4)	0.2 - 2.4 (1.3 ± 0.4)	2 - 59 (23 ± 5)

[a]Cobelo-García et al. (2005); [b]Santos-Echeandías et al. (2009); [c]Rodellas et al. (2014); [d,]Morillo et al. (2015); [e]Tovar-Sanchez et al. (2014a); [f]Tovar-Sanchez et al. (2014b); [g]Present study

Table 2.6 Average metal concentrations (Av) ± standard deviation (SD) (n = 3) and grain-size in surficial sediments from Maó Harbour. Metal concentrations are expressed in µg g-1, except Fe that is in % (dry weight).

Stations	Cd		Co		Cu		Fe		Mo		Ni		Pb		Zn		Grain size		
	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	Av	SD	<63 mm (%)	>63 mm(%)	
C1	nd	-	3,3	0,5	75,2	1,2	1	0	1,7	0,5	9,7	0,6	42,4	1,1	93,5	0,4	20	80	silty-sand
C2	nd	-	5,7	0,2	40,8	0,7	1,9	0	2,3	1,1	16,4	0,2	124	3	77,2	1,1	57	43	sandy-silt
C3	0,1	0,1	3,0	0,2	33	2	0,90	0	1,0	1,0	7,6	0,4	57	2	67	4	53	48	sandy-silt
C4	nd	-	0,85	0,14	2,1	0,2	0,2	0	1,1	0,6	1,4	0,6	14	2	17,1	0,8	23	77	silty-sand
C5	nd	-	4,5	0,4	48,0	0,6	1,6	0,1	2,6	1,0	16,2	0,3	93	6	91	2	35	65	silty-sand
C6	0,1	0,1	5,64	0,04	72	2	2,1	0,1	1,6	0,9	19,8	0,7	113	3	123	3	33	67	silty-sand

Av: average; SD: standard deviation

Table 2.7 Mean values ± standard deviation (SD) of metal concentrations in surficial sediments of different areas of Mediterranean Sea. Concentrations are expressed in µg g-1 (dry weight), except Fe (%).

Element	Gulf of Naples (Italy)[a]	Sfax Coast (Tunisia)[b]	Sardinia. Italy[c]	Corsica. France[c]	Toscana. Italy[c]	Ionian Sea. Southern Italy[d]	Maó Harbour[e]
Cd	0.57 ± 0.62	5.9 ± 0.5	0.07 ± 0.03	0.03 ± 0.00	0.40 ± 0.10	-	0.09 ± 0.00
Co	-	-	2.5 ± 0.0	55 ± 14	7 ± 1	-	5 ± 1
Cu	27 ± 29	16 ± 4	-	-	-	47 ± 3	65 ± 18
Fe	15 ± 13	4.9 ± 0.4	-	-	-	3.2 ± 0.3	1.9 ± 0.5
Mo	-	-	-	-	-	-	1.7 ± 0.2
Ni	7 ± 10	14 ± 8	4 ± 1	1 ± 5	40 ± 6	52 ± 3	18 ± 5
Pb	220 ± 170	32 ± 17	19 ± 2	5 ± 1	45 ± 5	57 ± 8	98 ± 25
Zn	600 ± 550	59 ± 17	-	-	-	100 ± 13	110± 30

[a] Romano et al., 2004; [b] Gargouri et al., 2011; [c] Lafabrie et al., 2007; [d] Buccolieri et al., 2006; [e] Present study.

Table 2.8 Average annual flows in mol y-1 of the different metal sources to the Maó Harbour. Numbers in parenthesis are the contribution in percentages of the total flow.

	Co		**Cu**		**Fe**		**Ni**		**Pb**		**Zn**	
Groundwater	13 ± 11	(2)	160 ± 70	(0.3)	1400 ± 900	(6)	160 ± 130	(3.7)	17 ± 23	(0.6)	600 ± 940	(0.7)
Stream waters	13 ± 12	(2)	160 ± 80	(0.3)	2000 ± 1500	(9)	180 ± 90	(4.1)	6 ± 3	(0.2)	210 ± 110	(0.3)
Atmospheric deposition*	1	(0)	10	(0)	1200	(5)	4	(0.1)	4	(0.1)	550	(0.7)
Sediments	620 ± 15	(96)	59500 ± 100	(99.4)	18600 ± 1700	(80)	4000 ± 160	(92.0)	3000 ± 20	(99)	76000 ± 33000	(98)
Export offshore	650		60000		23200		4400		3000		78000	

*Maximun value from Guieu et al, 1997

Table 3.2 Total cell abundance and cells expressing ELF (cells l-1) for main phytoplankton taxa over the 15 stations during June and October surveys. Other taxa within diatom and dinoflagellate groups account for small unidentified species in the corresponding group. The percentage (%) of individual species to total cells and to group abundance is also indicated. The methodological error is 20% and the estimated counting error is ±10%.

		Total		Total (%)				ELF- labeled		ELF (%)			
Group	Genus	Jun.	Oct.	Jun.		Oct.		Jun.	Oct.	Jun.		Oct.	
				% of total	% of group	% of total	% of group			% of total	% of group	% of total	% of group
Diatoms	*Asterionellopsis*	0	6862	0.0	0.0	12.0	15.5	0	31	0.0	0.0	0.5	23.8
	Chaetoceros	3932	29469	4.2	39.3	51.3	66.4	513	0	0.8	59.5	0.0	0.0
	Cyclotella	0	7919	0.0	0.0	13.8	17.8	0	31	0.0	0.0	0.5	23.8
	Licmophora	222	21	0.2	2.2	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Pseudo-nitzschia	208	26	0.2	2.1	0.0	0.1	0	21	0.0	0.0	0.3	16.2
	Rhizosolenia	439	10	0.5	4.4	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Striatella	739	0	0.8	7.4	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Thalassionema	901	0	1.0	9.0	0.0	0.0	0	0	0.0	0.0	0.0	0.0
	Thalassiosira	328	57	0.3	3.3	0.1	0.1	160	5	0.3	18.6	0.1	3.8
	Other	3230	42	3.4	32.3	0.1	0.1	189	42	0.3	21.9	0.6	32.3
Dinoflagellates	*Ceratium*	7140	373	7.5	8.4	0.6	3.2	2216	26	3.6	3.6	0.4	0.4
	Diplopsalis	2488	36	2.6	2.9	0.1	0.3	685	0	1.1	1.1	0.0	0.0
	Dinophysis	815	1575	0.9	1.0	2.7	13.6	197	204	0.3	0.3	3.0	3.0
	Prorocentrum	41711	840	44.1	49.3	1.5	7.3	35043	514	56.4	57.2	7.5	7.7
	Protoperidinium	4590	1155	4.9	5.4	2.0	10.0	3301	635	5.3	5.4	9.3	9.5
	Scrippsiella	12456	152	13.2	14.7	0.3	1.3	9281	105	14.9	15.1	1.5	1.6
	Other	15384	7448	16.3	18.2	13.0	64.3	10585	5221	17.0	17.3	76.3	77.8
Silicoflagellates		0	1423	0.0	0.0	2.5	100.0	0	10	0.0	0.0	0.1	100.0

Table 4.1 Average metal concentrations ± one standard deviation (SD), grain-size and total organic carbon (TOC) in surficial sediments from Mahon Harbour. Metal concentrations are expressed in µg g-1, except Ca, Al, Fe and TOC that are in % (dry weight).

	Ca (%)		**Sr**		**Al (%)**		**Fe (%)**		**Mn**		**Ni**		**Co**		**Pb**		**Zn**		**Cu**		**Hg**		**TOC (%)**	**Grain size**
	Av	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**	**Av**	**SD**		
S-3	7.9	0.5	419	16	0.8	0.2	1.0	0.0	106	4	9.7	0.6	3.3	0.5	42	1	93.5	0.4	75.2	1.2	0.30	0.01	1.1	Sandy-silt
S-4	11.2	0.9	603	122	1.8	0.1	1.8	0.0	172	4	16.4	0.2	5.7	0.2	124	3	77.2	1.1	40.8	0.7	1.43	0.04	0.8	Sandy-silt
S-2	17.9	1.0	1127	168	0.7	0.0	0.9	0.0	79	2	7.6	0.3	3.0	0.2	56	2	67.2	4.2	33.4	1.6	1.16	0.82	1.7	Sandy-silt
C-3	10.8	0.3	848	22	2.2	0.1	2.2	0.1	222	8	20.6	0.8	6.0	0.3	104	4	125.4	4.1	72.2	2.7	1.11	0.04	2.8	Silty
C-4	14.1	0.3	1068	19	2.4	0.0	1.9	0.0	209	2	16.4	0.2	5.3	0.1	89	2	111.6	3.4	59.0	1.3	0.87	0.04	2.2	Silty
S-5	26.3	1.0	1693	35	0.2	0.0	0.2	0.0	64	2	1.4	0.6	0.9	0.1	14	2	17.1	0.7	2.1	0.2	0.04	0.00	0.4	Silty-sand
C-5	11.6	0.2	879	13	2.1	0.1	2.2	0.1	225	5	21.2	0.3	6.0	0.3	109	1	131.1	2.5	77.2	4.3	1.22	0.01	2.9	Silty
C-7	13.6	0.3	1019	13	2.3	0.1	2.2	0.0	226	3	5.8	0.3	20.9	0.4	112	5	125.5	2.8	71.4	1.2	1.01	0.04	2.6	Silty
S-1	14.1	0.2	1048	15	1.8	0.3	1.6	0.0	181	5	16.2	0.3	4.5	0.4	93	6	91.2	1.8	48.0	0.5	0.84	0.03	1.6	Silty-sand
S-6	10.1	0.1	768	2	2.2	0.2	2.0	0.0	207	1	19.8	0.7	5.6	0.0	113	3	122.6	3.2	71.5	2.2	1.22	0.00	1.8	Silty-sand

Av: average SD:standard desviation

Table 4.2 Enrichment factor (EF), geoaccumulation index (Igeo) and actions limits level (CEDEX) for metal concentrations in surface sediments of obtained in this study.

	Average	**SD**	**EF**		**Igeo**		**Actions Limits (CEDEX)**
Fe	19313.45	0.50	1.76	Minor enrichment	-1.75	Uncontaminated	NA
Mn	201.15	47.22	1.10	Minor enrichment	-2.42	Uncontaminated	NA
Ni	18.10	5.00	1.27	Minor enrichment	-2.21	Uncontaminated	AL1
Co	5.33	1.24	0.87	No enrichment	-2.76	Uncontaminated	NA
Pb	97.65	25.03	25.94	Very severe enrichment	2.14	Moderately to strongly contaminated	AL1 - AL2
Zn	113.48	26.59	6.86	Moderately severe enrichment	0.22	Uncontaminated to moderately contaminated	AL1
Cu	64.67	17.55	10.17	Moderately severe enrichment	0.79	Uncontaminated to moderately contaminated	AL1
Hg	1.01	0.29	98.81	Extremely severe enrichment	4.07	Strongly to extremely contaminated	AL1 - AL2