frontiers in
MARINE SCIENCE

ORIGINAL RESEARCH ARTICLE
published: 18 December 2014
doi: 10.3389/fmars.2014.00079

Spatial gradients in trace metal concentrations in the surface microlayer of the Mediterranean Sea

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The relationship between dust deposition and surface water metal concentrations is poorly understood. Dissolution, solubility, and partitioning reactions of trace metals from dust particles are governed by complex chemical, biological, and physical processes occurring in the surface ocean. Despite that, the role of the sea surface microlayer (SML), a thin, but fundamental component modulating the air-sea exchange of materials has not been properly evaluated. Our study revealed that the SML of the Mediterranean Sea is enriched with bioactive trace metals (i.e., Cd, Co, Cu, and Fe), ranging from 8 (for Cd) to 1000 (for Fe) times higher than the dissolved metal pool in the underlying water column. The highest enrichments were spatially correlated with the atmospheric deposition of mineral particles. Our mass balance results suggest that the SML in the Mediterranean Sea contains about 2 tons of Fe. However, we did not detect any trends between the concentrations of metals in SML with the subsurface water concentrations and biomass distributions. These findings suggest that future studies are needed to quantify the rate of metal exchange between the SML and the bioavailable pool and that the SML should be considered to better understand the effect of atmospheric inputs on the biogeochemistry of trace metals in the ocean.

Keywords: sea surface microlayer, SML, trace metals, aerosol deposition, dust event, Mediterranean Sea

INTRODUCTION

The sea-surface microlayer (SML) has been defined as the top 1–1000 μm of the surface ocean and it serves as the boundary layer between the atmosphere and the ocean. The SML is known to concentrate natural and anthropogenic materials from the atmosphere as well as to be a micro-habitat for a diverse range of organisms (i.e., bacteria, phytoplankton, zooplankton, and icthyo-neuston) (Cincinelli et al., 2001). This environmental compartment plays an important role in the exchange rate of gases and energy on a global scale (Agogué et al., 2004; Wurl and Obbard, 2004; Wurl et al., 2006, 2011; Wurl and Holmes, 2008). It has been reported that metals are enriched in the SML mainly from atmospheric deposition and from rising bubbles, and can be influenced by organic matter concentrations, pH, and salinity, among other factors (Liss and Duce, 1997; Wurl and Obbard, 2004). While water-column trace metal concentrations, distributions and bioavailability in open ocean waters have been reported (e.g., Millero, 2006; Aparicio-González et al., 2012) SML studies are very limited and almost nonexistent for open ocean environments (Wurl and Obbard, 2004).

The Mediterranean Sea is a semi-enclosed basin with one of the highest rates of aeolian deposition in the world with strong pulses of mineral dust from Africa, in addition to consistent anthropogenic aerosol inputs from Europe (Bonnet and Guieu, 2006; Ternon et al., 2009; Jordi et al., 2012). The study of the interface between the ocean and atmosphere is therefore important to better understand trace metal distributions, as well as the processes influencing vertical fluxes of materials, phytoplankton uptake, and particulate scavenging occurring in surface waters of the Mediterranean Sea.

In this study, we established trace metal distributions in the SML and in the sub-surface water (<1 m) as well as the aerosol composition along a longitudinal transect from the western to the eastern parts of the Mediterranean Sea. The different Mediterranean marine regions were sampled twice within a single month both under high and low atmospheric deposition conditions. A network analysis was used to determine if any relationship exists between the various metal concentrations measured (SML, total and dissolved pools, and dust particles) and bacterial and/or phytoplankton biomass.

METHODS

Samples for this study were obtained on board the Spanish R/V BIO García del Cid during the cruise THRESHOLDS I, from June 4 to July 3, 2006. The concentrations of trace metals were measured in the SML, in the underlying water column, and in atmospheric particles collected in different regions of the Mediterranean Sea (i.e., Eastern and Western sections, Aegean, Marmara, and Black Seas). The cruise consisted of 2 transects sampling a total of 38 stations under different atmospheric

deposition conditions (Transect 1- stations 1–17, June 4–16, 2006 under low atmospheric deposition; and transect 2, stations 18–38; June 19 to July 3, 2006 under high atmospheric deposition) (**Figure 1**).

WATER SAMPLING AND ANALYSIS

SML and sub-surface water (SSW: collected to ∼1 m depth) samples were collected under calm sea conditions (average wind speed in the cruise: 3.7 ± 3.1 m s^{-1}) from a pneumatic boat deployed ∼2 km away from the research vessel to avoid metal contamination. For SML sampling (unfiltered water), we used a glass plate sampler (Stortini et al., 2012), which was acid cleaned overnight and rinsed thoroughly with MQ-water. In order to quantify any procedural contamination, we collected field SML blanks by rinsing and collecting 0.5 L of ultra pure water. The surface microlayer thickness (SML_T in μm) was calculated according to the formula of Wurl (2009):

$$SML_T = V_S \cdot 10^4 / N(A_p \cdot 2)$$

where V_S is the volume sampled (mL), N the number of dips, and A_p the surface area of the glass plate (cm^2). We used a 975 cm^2 surface area glass plate and about 100 dips were required to collect 500 ml of water at each station. With these parameters, we calculated a SML_T of 26 μm. Total (unfiltered) surface water samples (TSSW) were collected using an acid-washed Teflon tubing connected to a peristaltic pump. Dissolved water samples (DSSW) were filtered in-situ through an acid-cleaned polypropylene cartridge filter (0.22 μm; MSI, Calyx®) (Tovar-Sánchez, 2012). All water samples (TSSW, SML, and DSSW) were collected in 0.5 L acid cleaned low-density polyethylene plastic bottle and acidified on board to pH <2 with ultrapure-grade HCl in a class-100 HEPA laminar flow hood. Metals (Cd, Co, Cu, Fe, Mo, and V) were preconcentrated using an organic extraction method (Bruland et al., 1979), and quantified by ICP-MS (PerkinElmer ELAN DRC-e). The limit of detection, calculated as three times the standard deviation of the blank values ranged from 1 pM for Co to 0.6 nM for V. The accuracy of the metal analyses was established using Seawater Reference Material for Trace Elements (NASS5, NRCCNRC) with recoveries ranging from 85% for V to 100% for Cd.

Bacterial abundance was estimated by flow cytometry (Marie et al., 1997) and chlorophyll-a concentrations were determined fluorometrically (Parsons et al., 1984) using a Turner Design fluorometer after pigment extraction overnight with 90% acetone.

AEROSOL SAMPLING AND ANALYSIS

Coarse aerosol particles were collected on acid-washed cellulose filters (Whatman 41) using a high-volume particle collector (MCV: CAV-A/HF). The aerosol collector was mounted over the vessel bridge and connected to a wind direction sensor. In order to avoid contamination from the ship exhaust, the sampler automatically closed when wind direction deviated from the bow. Sampling flow rate was set at 40 m^3 h^{-1} and the sampling volumes ranged from 100 to 800 m^3 of filtered air. A microwave acid digestion procedure using HNO_3, HF, and H_2O_2 followed by ICP-AES (Perkin Elmer, Optima 5300 DV) quantification was used to measure total metal levels in the aerosol filters (Pekney and Davidson, 2005). The limit of detection for the particulate metal analyses ranged from 1.3 nM for Cd to 124 nM for Fe.

Bulk aerosol concentrations over the sampling area were calculated using the daily L3 level estimates from the MODerate Resolution Imag-ing Spectroradiometer (MODIS) satellite data (http://gdata1.sci.gsfc.nasa.gov/daac-bin/G3/gui.cgi?instance_id=MODIS_DAILY_L3).

ASSOCIATION NETWORKS

The association networks between the metal concentrations measured in the Mediterranean Sea (aerosols and water column) with other parameters were constructed by computing the Pearson

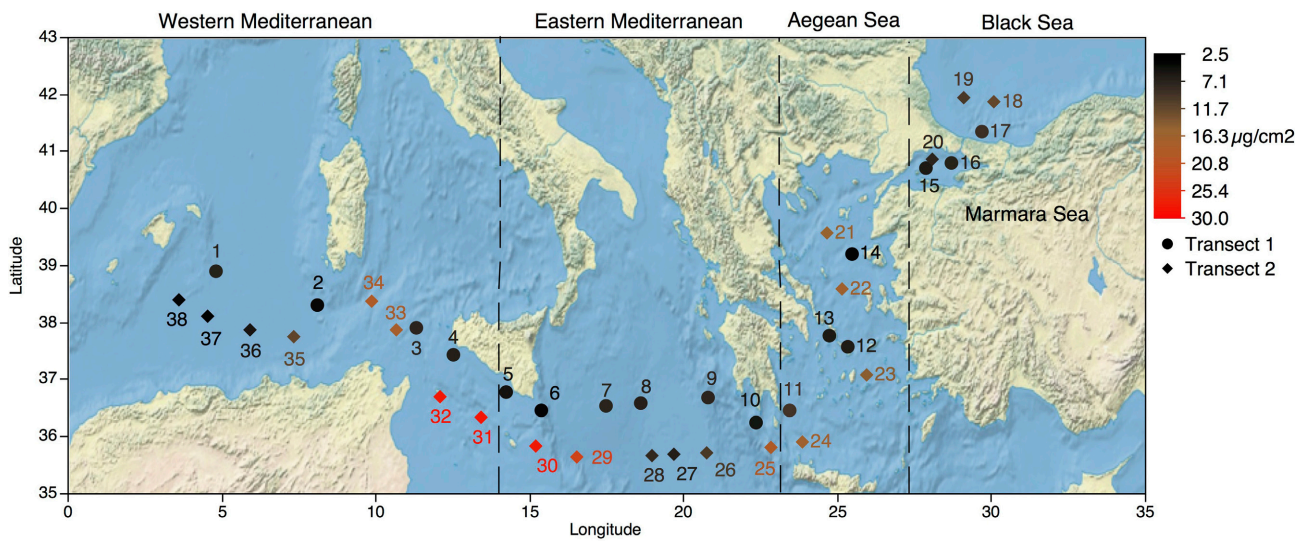

FIGURE 1 | Location of the sampling sites along the Mediterranean Sea. Color scale indicates bulk aerosol concentrations from satellite observations (MODIS).

correlation coefficient between each set of variables. Only positive correlations were used in the analysis and the resulting networks were plotted with the Cytoscape software environment program (Shannon et al., 2003), using the Pearson product-moment correlation coefficients as weighting variables of the "edge-weighted spring embedded" layout.

RESULTS AND DISCUSSION

SURFACE WATER CONCENTRATIONS AND LONGITUDINAL DISTRIBUTIONS OF TRACE METALS

Metal concentrations varied broadly among the three water column reservoirs and exhibited a strong spatial variability within the Mediterranean basin (**Table 1** and **Figure 2**). High concentrations of Cd, Co, Cu, and Fe in the DSSW pool were measured in the Marmara and Black Seas (e.g., mean DSSW ± SD averaged from both Seas; 91 ± 19 pM, 366 ± 138 pM, 7.6 ± 1.1 nM and 3.4 ± 1.2 nM, respectively). In contrast, levels of Mo and V in the three pools (SML, TSSW, and DSSW) in the Marmara and Black Seas were about 50% lower (e.g., mean DSSW ± SD; 50.6 ± 9.1 nM and 10.8 ± 2.9 nM, respectively) than those measured in other regions of the Mediterranean Sea (120.6 ± 8.1 nM and 25.7 ± 2.6 nM, respectively). This is consistent with the geochemistry of the Marmara and Black Seas where high metal levels have been linked to anthropogenic inputs and river discharges (OGUZ, 2005; Okuş et al., 2007). In contrast, particle scavenging in the anoxic or near-anoxic waters of the Black Sea seems to be responsible for the low Mo and V concentrations (Emerson and Huested, 1991). This metal removal is consistent with the low oxygen saturation levels measured in the Black (average: 28 ± 4%) and Marmara Seas (average: 34 ± 2%), in contrast to the 74 ± 2% calculated for the rest of the sampling locations along the Mediterranean. The increased nutrients and metals inputs to the Black Sea have induced a basin-scale eutrophication (Chu et al., 2005) which is reflected in the high chlorophyll-a concentration measured in those waters (i.e., 1.7 ± 0.2 mg m^{-3}). Although DSSW metal concentrations were less variable in the other regions of the Mediterranean Sea, higher levels ($p < 0.005$) of Cd (97 ± 13 pM), Co (169 ± 61 pM), and Cu (2.7 ± 0.9 nM) were measured in the Aegean Sea, in comparison to the concentrations detected in the Eastern (Cd: 79 ± 6 pM, Co: 115 ± 20 pM, and Cu: 1.6 ± 0.2 nM) and Western (Cd: 70 ± 9 pM, Co: 95 ± 30 pM, and Cu: 1.6 ± 0.3 nM) Mediterranean. Mean Fe, Mo, and V concentrations in the DSSW pool were similar ($p < 0.005$) in these three regions (i.e., Aegean Sea: 3.0 ± 0.7 nM, 117.0 ± 9.9 nM, 24.6 ± 3.7; Eastern: 3.1 ± 1.4 nM, 123.0 ± 4.3 nM, 25.7 ± 1.6 nM, and Western: 2.8 ± 1.7, 120.7 ± 9.2 nM, 26.3 ± 2.4 nM). These concentrations are consistent with the levels previously reported for the Mediterranean Sea (Lewis and Landing, 1992; Vega and van den Berg, 1997; Yoon et al., 1999).

Metals with a strong affinity for organic ligands (i.e., Cd, Co, Cu, Fe) (Bruland and Lohan, 2006) were enriched in the SML, 8, 41, 16, and 1000 times higher respectively, over the total metal fraction measured in the subsurface water (TSSW) and 12, 55, 20, and 4700 times over the dissolved fraction (DSSW) (**Table 1** and **Figures 2**, **3**). These metal enrichments are consistent with the high levels of organic matter and surfactants found in the SML (Buck et al., 2010; Wurl et al., 2011). Less reactive elements such

Table 1 | Bacterial abundance, density of particles in the atmosphere from satellite observation (MODIS), chlorophyll-a and trace metals concentrations in the different environmental compartments (i.e., SML, Surface microlayer; TSSW, Total subsurface water; DSSW, Dissolved Subsurface Water; and aerosols coarse particles).

Station	Date (2006)	Latitude	Longitude	Sea Section	Chl *a* mg m^{-3}	SML						
						Cd, pM	Co, pM	Cu, nM	Fe, nM	Mo, nM	V, nM	BA, cell mL^{-1}
1	June 04	38.90333	4.80250	WesternM	0.29	312	276	9.6	155.0	144.2	37.9	905635
2	June 05	38.30867	8.09500	WesternM	0.34	224	799	14.7	223.1	140.8	35.0	842582
3	June 06	37.91017	11.31117	WesternM	0.27	171	402	9.5	354.2	124.6	29.1	566838
4	June 06	37.43700	12.51467	WesternM	0.26	798	533	5.4	354.7	132.0	29.6	668539
5	June 07	36.78267	14.22863	WesternM	0.25	225	467	7.9	834.2	115.2	31.9	783568
6	June 07	36.46167	15.37083	EasternM		92	47			128.0	25.8	674530
7	June 08	36.54067	17.47232	EasternM	0.23	375	35	1.2	62.6	122.0	26.7	
8	June 08	36.58867	18.60100	EasternM	0.21	191				123.1	22.3	598323
9	June 09	36.68650	20.78668	EasternM	0.21	17	54		20.0	118.4	25.2	
10	June 12	36.24930	22.34617	EasternM	0.21	161	61	3.3	116.4	130.2	27.7	774216
11	June 12	36.46150	23.43783	Aegean Sea		53				121.8	26.6	682945
12	June 13	37.57750	25.32600	Aegean Sea	0.30							
13	June 14	37.77283	24.72767	Aegean Sea	0.13							515734
14	June 15	39.20482	25.46500	Aegean Sea	0.22	121	1033	17.4	226.7	150.3	37.6	691314
15	June 15	40.71282	27.86467	Marmara Sea	1.04	79	374	9.4	55.4	62.5	14.9	723067
16	June 15	40.80332	28.69200	Marmara Sea	1.88	1084	675	24.1	566.1	58.7	14.7	
17	June 16	41.35098	29.68382	Black Sea	1.49	154	113	6.8	25.2	45.2	10.2	1304266
18	June 19	41.87223	30.07232	Black Sea	1.90	157	1129	23.9	2799.1	39.4	15.8	
19	June 20	41.93517	29.09182	Black Sea	1.71	116	168	11.8	70.0	42.8	8.1	
20	June 21	40.86417	28.05817	Marmara Sea	3.78	225	313	11.5	134.1	55.9	10.3	1017006
21	June 22	39.57750	24.65887	Aegean Sea	0.19	70	438	12.1	225.9	127.9	29.4	579521
22	June 23	38.58850	25.16100	Aegean Sea	0.21	44	125	41.2	243.6	131.6	29.3	695139
23	June 23	37.09000	25.92450	Aegean Sea	0.11	85	126	10.9	167.1	127.7	26.6	519674
24	June 24	35.90717	23.87040	Aegean Sea	0.22	41	397	5.2	963.4	125.4	28.7	
25	June 24	35.81267	22.82522	EasternM	0.17							
26	June 25	35.71582	20.73850	EasternM	0.20	45	274	2.8	684.9	131.9	29.3	522692
27	June 26	35.70500	19.69432	EasternM	0.20	13		1.0	70.6	117.7	22.0	662607
28	June 27	35.67332	18.95447	EasternM	0.16	8		0.3	64.7	159.0	14.7	676380
29	June 27	35.65650	16.53482	EasternM	0.21	45	4456	29.6	9023.8	105.2	56.5	598286
30	June 27	35.83700	15.20797	EasternM	0.18	26		6.2	13.5	129.9	26.3	
31	June 30	36.34650	13.39217	WesternM	0.21							596637
32	June 30	36.71232	12.06300	WesternM	0.23	63		3.5	169.7	113.8	22.7	
33	July 01	37.88457	10.64432	WesternM	0.28	67	2	2.5	77.4	146.0	29.7	
34	July 01	38.37945	9.84361	WesternM		95	22	11.5	117.3	121.1	24.9	648900
35	July 02	37.76267	7.34350	WesternM	0.21	29		3.5	174.6	125.2	26.2	705061
36	July 02	37.87243	5.92632	WesternM	0.22	22		0.9		119.7	21.7	756314
37	July 03	38.12117	4.53282	WesternM	0.29				153.5	128.9	31.6	712789
38	July 03	38.40582	3.58832	WesternM	0.31		24		12.6	141.4	30.7	837003

Station	TSSW							DSSW						Aerosols (pmol.m^{-3})				MODIS
	Cd, pM	Co, pM	Cu, nM	Fe, nM	Mo, nM	V, nM	BA, cell mL^{-1}	Cd, pM	Co, pM	Cu, nM	Fe, nM	Mo, nM	V, nM	Cu	Fe	Mo	V	µg cm^{-2}
1							780720							47.8	3.1	11.1	BDL	6.91
2							739107	64	100	1.2	2.4	119.4	26.2	41.2	2.6	55.5	BDL	4.22
3							699800	63	45	1.1	1.6	107.1	23.4					7.34
4							689216	68	81	1.3	2.3	117.6	28.2					6.46
5		164	1.8	4.0	139.7	35.4		74	88	1.5	2.0	133.0	30.4					4.95
6							661304	72	134	1.6	6.1	115.1	21.7					3.78
7								74	93	1.7	2.2	120.5	26.3					7.07
8		222	2.0	5.2	152.2	39.2		82	125	1.4	1.9	125.2	27.0	77.5	1.3	3.3	11.6	6.72
9		218	2.4	7.1	142.4	32.8		78	133	1.6	3.3	123.6	25.7	44.9	0.8	32.3	191.7	7.32
10	84	137	2.1	11.2	128.8	28.7	645180	73	107	1.6	2.6	121.2	25.6					5.19
11			2.4	5.5	124.2	25.6	644288	92	125	1.9	2.1	113.8	25.4	70.4	3.4	18.9	214.8	9.18
12								109	157	3.4	3.4	112.0	23.1	56.6	4.5	3.5	11.2	6.36
13							621366	101	207	2.8	2.8	126.2	27.4	22.2	1.9	BDL	BDL	5.80
14	87		2.0	5.0			601143	80	121	2.0	2.6	129.6	29.5					3.81
15	111	489	9.5	13.8	58.9	13.1	881789	107	451	7.7	3.7	55.2	11.2					5.92
16	128	488	9.1	21.3	59.3	12.7		110	466	8.0	4.4	58.6	11.3					6.77
17	91	195	6.9	7.5	43.0	8.8	1154218	87	182	6.0	1.8	39.4	6.2					8.51
18	96	290	7.8	5.5	44.7	10.6		80	217	6.7	2.0	42.1	8.7					11.63
19	85	298		10.7				60	368	8.8	3.7	46.9	14.3					9.71
20	124		8.8	14.9			854627	98	515	8.6	4.9	61.3	13.0					7.30
21		407	5.2	36.6			629914	117	291	4.4	4.0	105.3	19.0					15.12
22		216	2.6	8.1	136.9	24.8	609771	94	116	2.0	2.2	106.3	20.7					16.21
23	81		2.1	6.3	130.7	28.8	583904	80	127	1.8	3.5	128.9	28.1	123.3	15.0	14.4	195.6	14.29
24				5.1		23.7		104	133	3.3	3.1	114.0	23.5					16.37
25	93	157	2.3	9.5				89	128	1.9	5.7	127.0	26.5	130.2	19.6	30.9	401.5	18.81
26	88	130	2.0	17.1			645299	85	123	1.6	2.7	121.7	25.7					9.76
27		187	2.2	6.7	148.2	34.2	649614	89	103	1.6	3.2	122.8	25.9					6.58
28			2.1	5.7		26.9	650365	79	143	1.7	2.3	130.4	26.2					7.87
29	80	108	1.9	8.6			672231	75	81	1.5	1.9	118.9	24.7	48.2	26.2	75.2	743.8	23.44
30	80	126	1.9	7.5				71	101	1.8	2.5	126.9	28.0	60.6	46.7	106.0	961.2	27.92
31		116	1.8	10.1	129.9	27.0	685789	82	95	1.6	8.0	121.2	25.3					28.68
32	85	105	1.9	9.4				79	88	1.8	2.8	115.7	25.1	86.8	21.3	91.5	BDL	28.21
33	78	109		9.0				76	105	1.7	2.3	120.3	25.0	82.1	14.6	BDL	150.4	17.45
34		101	2.0	18.6			737387	80	70	1.5	2.5	118.2	26.0					18.46
35			1.6	7.0	119.6	25.8	750065	75	87	1.4	2.0	113.6	22.6	158.9	8.1	33.4	3.0	11.31
36	68			4.0		24.2	776244	65	130	2.3	2.6	120.4	25.7					5.49
37	61	126	2.5	6.4	126.8	28.3	783285	58	91	1.3	1.9	118.7	27.3	304.5	13.1	9.4	285.6	3.12
38	77	105		7.9			797146	58	166	2.1	2.7	142.9	30.2					3.69

Aerosols in pmol m^{-3} except Fe (in nmol m^{-3}), Cd and Co concentrations in aerosols particles were not detected; Empty cells indicate not measured values. BLD, Below the limit of detection.

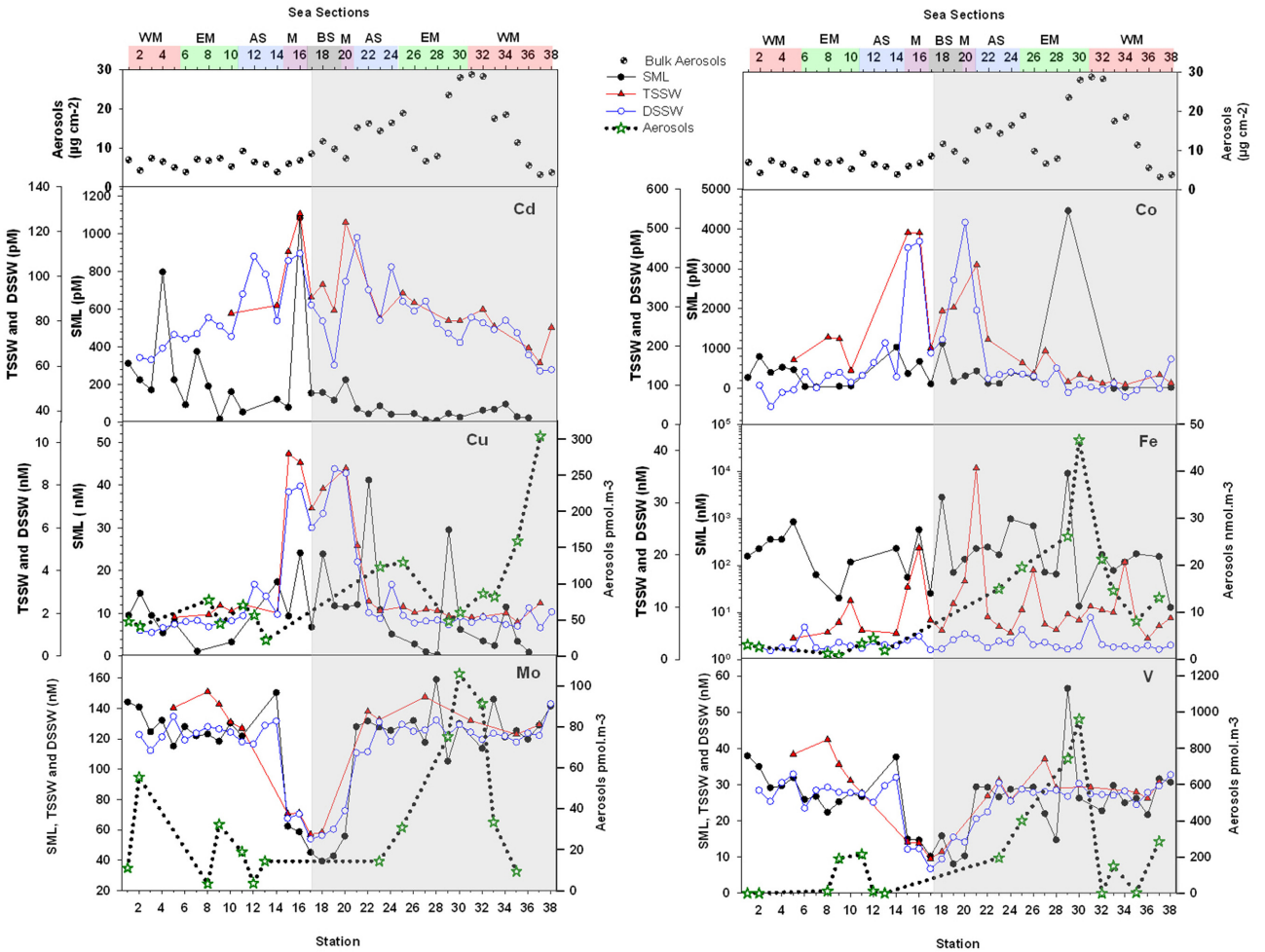

FIGURE 2 | Bulk aerosol and metal concentrations measured in the different water-column compartments. SML, surface micro-layer: black dots; TSSW, total subsurface water: red triangles; DSSW, dissolved subsurface water: open blue dots; and aerosols, black-white dots. Shaded area represents the second transect. Shaded colors in the upper x-axis shows stations in different regions of the Mediterranean Sea: WM (Western Mediterranean), EM (Eastern Mediterranean), AS (Aegean Sea), M (Marmara Sea), and BS (Black Sea).

as Mo and V showed comparable concentrations among the different water compartments. The metal composition of the SML has not been extensively studied and different sampling protocols which influence the thickness of the sampling micro-layer, make any comparison with results obtained in other studies difficult. Despite that limitation, the SML metal enrichments calculated for the Mediterranean Sea were in good agreement with values reported elsewhere (Wurl and Obbard, 2004).

CONTRIBUTION OF AEROSOL INPUTS TO TRACE METAL CONCENTRATIONS AND DISTRIBUTIONS IN THE SEA SURFACE MICRO LAYER

During our second transect, the Eastern and Western regions of the Mediterranean were influenced by an African aerosol dust deposition event (**Figures 1**, **2**). However, aerosols back-trajectories (**Figure 4**) showed that the Mediterranean was also influenced by atmospheric particles originating from other different sources: the Aegean Sea (stations 21–24) and Western regions (stations 31–34) were receiving aerosols from European sources while the atmospheric particles deposited in the Eastern Mediterranean were from northern Africa (stations 29 and 30). Despite the back-trajectory results, the MODIS information showed that atmospheric particle densities higher than the background of 10 μg cm^2 were only detected in the Aegean Sea and in the Eastern and Western regions of the Mediterranean Sea (**Figure 2**).

Large-sized dust particles are responsible for most of the dry deposition measured in the ocean (Chester et al., 1999; Koçak et al., 2007), with Fe mostly found in the coarse fraction of the mineral aerosols (Duce et al., 1991). In our study, the highest aerosol density obtained from the satellite observations was detected between stations 30 and 32 (range 27.9–28.7 μg cm^{-2}) and those results matched with the highest concentrations of Fe, Mo, and V (46.7 nmol m^{-3}, 106.0 pmol m^{-3}, 961.2 pmol m^{-3}, respectively) measured in the same area during the second transect of our cruise (**Figure 2** and **Table 1**). The high

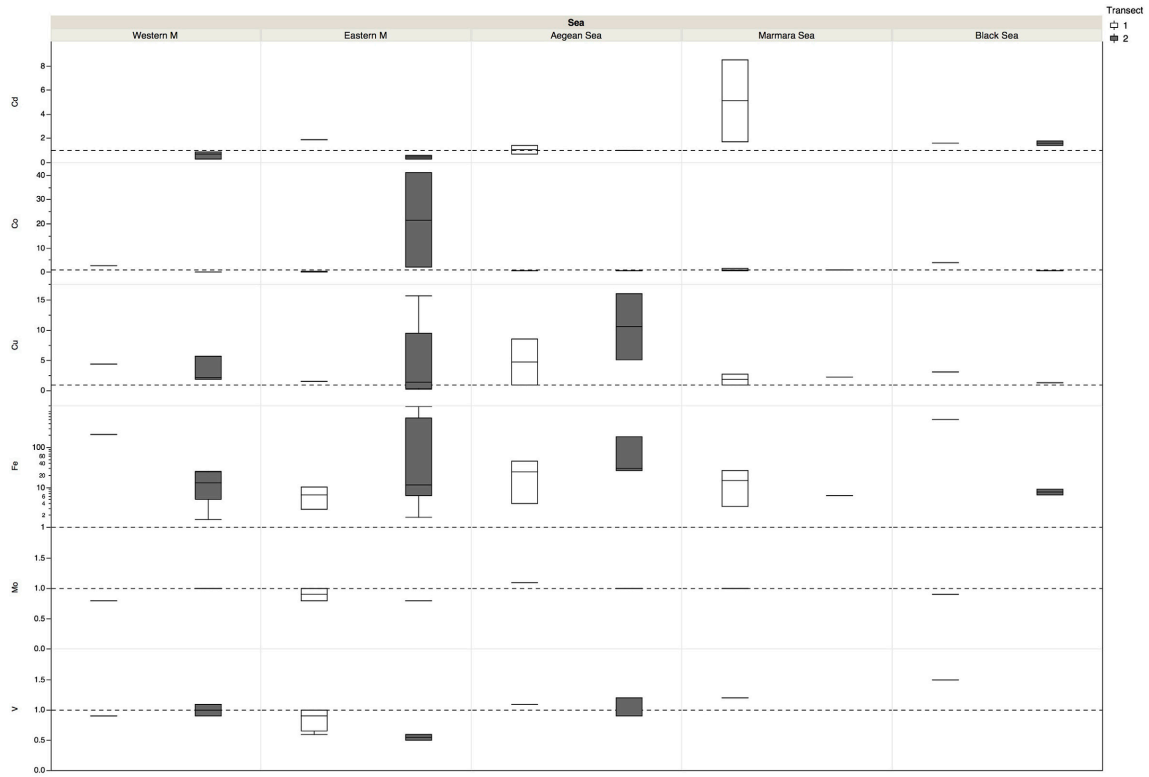

FIGURE 3 | Enrichment factors calculated for Cd, Co, Fe, Mo and V in the SML with respect to the subsurface seawater. Dashed line indicates an enrichment factor of 1.

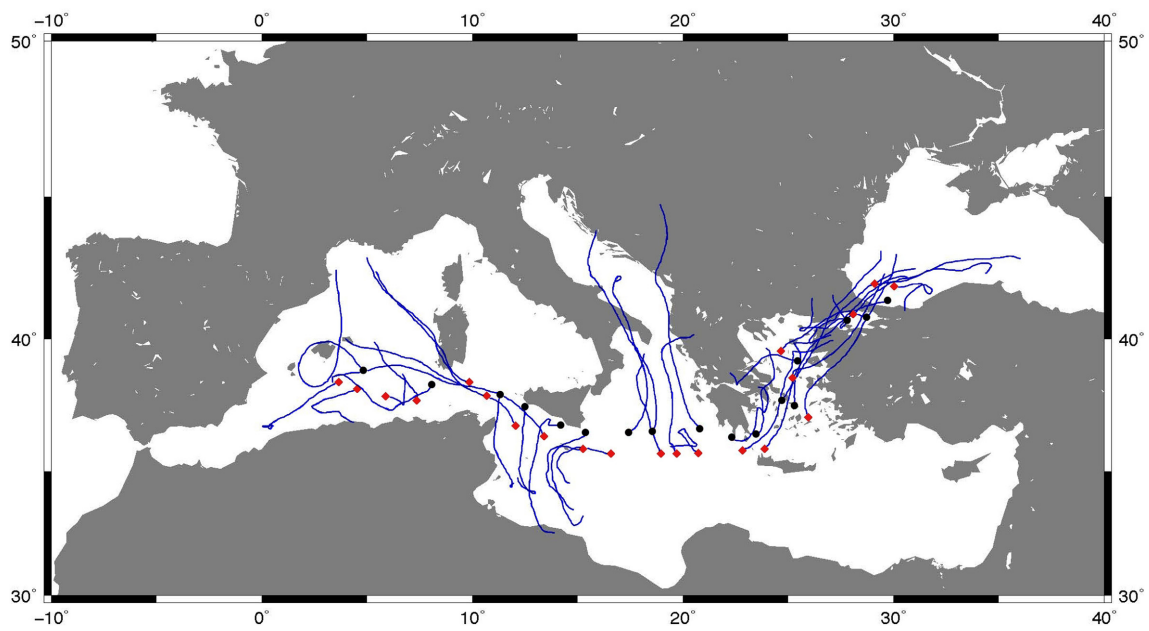

FIGURE 4 | Forty-eight-hour integrated backward trajectory of air masses reaching the sampling sites in the Mediterranean Sea (Transect 1: black dots; transect 2: red triangles). Air mass trajectories were calculated using the NOAA Hybrid Single-Particle Lagrangian Integrated Trajectory Model (HYSPLIT) available at http://ready.arl.noaa.gov/HYSPLIT_traj.php.

concentrations of Fe, V, and Mo suggest a mixture of natural and anthropogenic particles as Mo and V have been used as tracers for oil combustion (Addison et al., 1986; De Foy et al., 2012). While Cd and Co in our coarse aerosols samples were below our detection limits, Cu showed the highest concentration in the westernmost stations, also influenced by anthropogenic aerosols from Europe (Jordi et al., 2012) (**Figure 4**). Cadmium and Co were not detected in our samples suggesting that those two elements must be associated with the fine fraction of the mineral aerosols.

The concentration of Fe in the SML was punctually enhanced during the high aerosol dust event that occurred during the second transect of our cruise (**Figure 2**). The concentration of Fe in the aerosols increased, on average 10-fold (but up to 30 fold in the eastern sector) in the 7–26 day interval between the two transects (from 2.4 $\pm$ 1.4 nmol m^{-3} in the first transect, in stations 2–14 to 23.9 $\pm$ 12.0 nmol m^{-3} during the dust event in the second transect, in stations 21–34). The concentration of Fe in the SML at the same stations experienced a parallel increase, from 274.0 $\pm$ 257.9 to 985.1 $\pm$ 2547.3 nM between the two transects. However, the high averages calculated during the second transect are influenced by the high concentrations measured in station 29 (9024 nM) and stations 24 and 26 (963 and 685 nM, respectively). Despite this Fe increase in the SML, the high aerosol input was only slightly reflected in the TSSW pool (from 6.3 $\pm$ 2.6 nM to 11.3 $\pm$ 8.2 nM) and not detectable in the DSSW where concentrations remained relatively constant in both transects (from 2.7 $\pm$ 1.2 nM to 3.4 $\pm$ 1.6 nM) (**Figure 2**). This finding suggests that although most of the aerosol Fe in the Mediterranean is contained within the SML, this Fe pool does not exchange very fast with the DSSW fraction where most of the bioavailable Fe is found. These results are consistent with the uncoupling between dust deposition events and the vertical export flux of particulate metals recently reported for the Western Mediterranean (Heimbürger et al., 2014) and the lack of a link between dust supply and a rapid biological response (Eker-Develi et al., 2006). While Mo and V were also enriched (3–5 times) in aerosols particles during the dust event (stations 21–34), this increase did not coincide with in a similar enrichment in the SML probably as a consequence of the low reactivity of oxyanions in seawater (**Figures 2**, **3** and **Table 1**).

THE ROLE OF THE SML IN THE METAL BUDGET OF THE MEDITERRANEAN BASIN

A simple mass balance was used to determine the relative importance of the SML pool to the metal budget of the Mediterranean basin. With a surface area of 2.5 10^6 km^2, an average concentration of Fe in our survey (excluding the enclosed basins of the Marmara and Black Seas) and a SML thickness of 26 μm (see method section), we estimated that SML of the Mediterranean Sea contains about 2.2 tons of Fe. This amount represents between 0.2 and 0.5% of the TSSW and DSSW pools of Fe over the top meter of the surface sea, respectively (1289 and 412 tons of Fe in the TSSW and DSSW, respectively). Nevertheless, our results also showed that the percentage of the Fe pool contained in the SML could account for up to 3 and 12% of the TSSW and DSSW pools in the top-meter of the surface layer, respectively, during an episodic dust event (e.g., station 29 in the Eastern Mediterranean during the second transect). The total amount of metals, other than Fe, found in SML was only <0.15% of the mass found in the TSSW and DSSW pools. However, our mass balance estimates were carried out for the whole Mediterranean Sea and therefore, the relative contribution of the SML to the total metal pool of other smaller basins within the Mediterranean Sea could be significantly higher. Nevertheless, our analyses suggest that the SML must be considered to better constrain the potential effect of atmospheric inputs of some trace metals (e.g., Fe) on biological processes in the sea.

ASSOCIATION NETWORK ANALYSIS

An association network(s) analysis based on significant positive correlation analysis was used to visualize relationships among the different metals pools and their influence on bacterial abundance and phytoplankton biomass along our cruise. This analysis was carried out by pooling all the data generated during each transect of the cruise (**Figure 5A**: Transect 1 and **Figure 5B**: Transect 2). The results of the first cruise analysis, during low aerosol load conditions, showed two distinctive clusters (**Figure 5A**): a cluster caused by the correlations observed for the oxyanions, Mo and V in the different water column pools (SML, TSSW, and DSSW); and a second cluster formed between the concentrations of Cd, Co, Cu, and Fe measured in the dissolved and total pools (DSSW and TSSW) with the biological parameters (biomass of phytoplankton, as chlorophyll-a, and bacteria abundance in the SML and in the underlying water). This second cluster suggests that the bioavailable pool of trace metals was not in the SML but in the dissolved fraction. Except for V, no relationship among metal concentrations in any of the water compartments (SML, DSSW, and TSSW) and their concentrations in aerosols was observed. The correlation of V with the total amount of dust particles suggest that under low dust conditions, the chemical composition of the aerosols collected along the Mediterranean was influenced by anthropogenic sources (De Foy et al., 2012), although this correlation was biased by the high levels of this element measured at stations 9 and 11 in the Eastern Mediterranean and Aegean Sea (**Figure 2**). This analysis also showed that the distribution of metals in the SML during the first transect of our cruise was independent of dust concentrations as well as the TSSW and DSSW pools. The second cruise results also showed two clusters (**Figure 5B**). However, during this cruise the effect of the dust deposition event was more pronounced, as indicated by the correlation between the total amount of dust particles (reported as MODIS_Aero) with the actual Fe and Mo concentrations measured in the collected dust. As observed in the first transect of our cruise, biological parameters were independent of the SML metal composition.

Our results suggest that the biological response, as well as the concentrations of total and dissolved pools of active elements in the subsurface layer, are not immediately influenced by the metals present in the SML. However, this metal compartment might act as trap for aerosols particles and the retention capacity of the SML as well as the transfer rate to other reservoirs is poorly understood and needs to be evaluated in future studies.

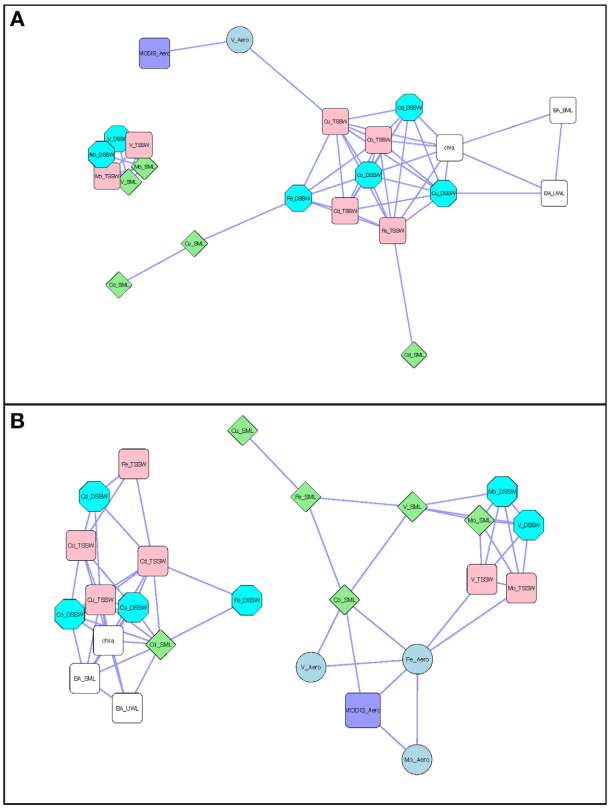

FIGURE 5 | Network analysis results obtained for transect 1 (A) and transect 2 (B). Elements in the surface microlayer (SML): Cd_SML, Co_SML, Cu_SML, Fe_SML, Mo_SML and V_SML; elements in the unfiltered fraction of the subsurface water (TSSW): Cd_TSSW, Co_TSSW, Cu_TSSW, Fe_TSSW, Mo_TSSW and V_TSSW; Elements in the dissolved fraction of the subsurface water (DSSW); Cd_DSSW, Co_DSSW, Cu_DSSW, Fe_DSSW, Mo_DSSW and V_DSSW; Elements in aerosols particles (Aero): Fe_Aero, Mo_Aero and V_Aero; Density of particles in the atmosphere from satellite observations (MODIS_Aero); chlorophyll-a concentrations (chla); and bacterial abundance in the SML (BA_SML) and in the underlying water column (BA_UWL). Nodes (SML: green diamonds; TSSW, red squares; DSSW, cyan octagons; Aero: blue circles; MODIS_Aero, purple square; chla and Ba_SML and Ba_UWL: white squares) correspond to the measured concentrations or abundances of the variables and edges represent a significant ($p < 0.05$) positive correlation between the two variables linked.

CONCLUSION

The Mediterranean Sea SML is enriched in reactive metals, such as Cd, Co, Cu, Fe over the metal fractions measured in the subsurface water. We estimated that the SML of the Mediterranean Sea contains a significant amount of Fe. However, the effect of bioactive trace metals found in the SML on the biological activity of the Mediterranean Sea was not immediately observed in our network analysis. These findings are particularly important for understanding the biogeochemistry of the Mediterranean Sea where atmospheric inputs are the main source of metals to the open waters of this important marine region (Migon, 2005).

ACKNOWLEDGMENTS

This work was supported by the project THRESHOLDS (CTM2005-24238-E) funded by the EU-FP6. We thank the crew of BIO García del Cid and the rest of the THRESHOLDS participants for cooperative work at sea. We also thank A. Massanet for technical assistance and J. F. González (Serveis Cientificotècnics, UIB) for technical support with the ICP-MS and ICP-AES.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 09 October 2014; accepted: 02 December 2014; published online: 18 December 2014.

*Citation: Tovar-Sánchez A, Arrieta JM, Duarte CM and Sañudo-Wilhelmy SA (2014) Spatial gradients in trace metal concentrations in the surface microlayer of the Mediterranean Sea. Front. Mar. Sci. **1**:79. doi: 10.3389/fmars.2014.00079*

This article was submitted to Marine Biogeochemistry, a section of the journal Frontiers in Marine Science.