

## ***Interactive comment on “Characterising the surface microlayer in the Mediterranean Sea: trace metals concentration and microbial plankton abundance” by Antonio Tovar-Sánchez et al.***

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We really thank the reviewers for their constructive comments. We believe that this new version of the manuscript has been improved significantly by the reviewers' suggestions as we have addressed all the points they raised. Generally, the text has been modified and we believe it has significantly improved. Specifically, grammatical and syntactic mistakes have been corrected and in general the text has been grammatically reviewed by an English native speaker. We have incorporated further clarification in all section of the manuscript; we have modified tables and figures, incorporated new references and elaborated new figures and tables. The response and actions taken to accommodate

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the reviewers' comments are described in the following pages.

**Reviewers' Comment:** This manuscript contains the measurement of many ancillary and biological parameters and trace metal concentrations in the surface microlayer and immediate underlying water collected during a Mediterranean cruise that covered all the main basins of the western and middle Mediterranean Sea. The manuscript is a fine effort in shedding light in the description of this microenvironment and the parameters that can affect its special biochemical characteristics. Despite its importance for interface processes, not many efforts are dedicated to the surface microlayer and this work is addressed to partially cover this deficit. Due to the amount of work involved and the relevance of the work for the common readers of Biogeosciences I think that the manuscript is well suited for its publication in this journal. The manuscript is well organized although it is obvious that more than one researchers have taken care of different parts, not all of them showing the same skill to write scientific English. Some parts will require grammar revision before publication. I would also miss that they present more data in the text since as it is the reader has to be continuously going back and forth to the tables and those are not reader friendly due to their size. Overall, I would back a major revision decision; the database presented here is very interesting and many parts of the interpretation are very useful but I think that the manuscript can be substantially improved in many aspects.

**Authors' Response:** We are very grateful by the deeply review and comments made by this referee, we believe that his/her comments and suggestions have helped to improve significantly the manuscript. The manuscript has been revised grammatically by a native English speaker. We have included more data during the discussion in the different sections of the manuscript.

**Reviewers' Comment:** Before publication, I have three major concerns that the authors need to address: Photoreactions. In a layer so exposed to solar radiation and with a heavy presence of organics prone to form radicals, the authors should have a better understanding of how these processes can affect species distribution in the SML

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and fluxes off it. However, these reactions are only invoked when the authors cannot explain, with their limited battery of processes, the distribution of a particular trace element. Just as a last resource. And I want to underline that not all metals are equally prone to those effects. It is well known the strong dependence of Cu and Fe redox seawater chemistries on solar radiation. Under strong solar radiation it is very likely that most of Cu and Fe are present as Cu(I) and Fe(II). Then the regular chemistry in seawater shifts, Cu(I) is a weaker acid and binds preferentially weaker acids (S-2) and Fe(II) is far more soluble (6 orders of magnitude!!!) and forms weaker complexes than Fe(III). I have to accept that not much is known about the speciation (organic and redox) of trace elements in the SML but the authors should try to gather all information available and use it for interpretation. Surprisingly, solar radiation is claimed to play a role in Ni speciation, a metal that is not likely to experience redox changes in seawater conditions (page 13, 15-18). I suggest a better compilation of bibliography referred to photochemical reactions of trace metals in surface waters, clearly identify those metals that can suffer redox reactions and apply this knowledge to the interpretation of distributions from the introduction and not as a last resource.

Authors' Response: We agree with the reviewer on the importance that the solar radiation has on the redox chemistry of the highly particle reactive elements, such as Fe and Cu. We have now included some discussion on this topic in the manuscript (section 3.2.1). We are also aware that Ni in seawater is thought to occur partly as stable organic complexes and with dissociation rates of its complexes much lower than Fe or Cu (104 times lower in the case of Cu; Morel and Hering, 1993). However, we believe that this dissociation rate could be significantly accelerated by the photochemical reactions and therefore directly affecting its speciation distributions and biological uptake and response. Although the interactions of Ni with dissolved organic matter have not been well studied in seawater, it is thought to occur partly as stable organic complexes and with slow dissociation rates (eg. Jiann et al., 2005; Wen et al. 2011 and reference therein). However, it is known that intense UV radiation can alter concentration, structure, reactivity and metal binding capacity of the organic matter increasing the

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proportion of free metals ion and their bioavailability and/or potential toxicity (Cheloni and Slaveykova, 2018). We have included this discussion in the text (section 3.2.4).

- Jiann, K.-T., Wen, L.-S., Santschi, P.H., 2005. Trace metal (Cd, Cu, Ni and Pb) partitioning, affinities and removal in the Danshuei River estuary, a macro-tidal, temporally anoxic estuary in Taiwan. *Marine Chemistry* 96, 293-313. - Morel, F.M.M., Hering, J.G., 1993. *Principles and Applications of Aquatic Chemistry*. Wiley, New York, p. 400. - Wen, L.-S., Santschi, P. H., Warnken, K. W., Davison, W., Zhang, H., Li, H.-P. and Jiann, K.-T.: Molecular weight and chemical reactivity of dissolved trace metals (Cd, Cu, Ni) in surface waters from the Mississippi River to Gulf of Mexico, *Estuarine, Coastal and Shelf Science*, 92(4), 649–658, doi:10.1016/j.ecss.2011.03.009, 2011. - Cheloni, G. and Slaveykova, V.: Combined Effects of Trace Metals and Light on Photosynthetic Microorganisms in Aquatic Environment, *Environments*, 5(7), 81, doi:10.3390/environments5070081, 2018

Reviewers' Comment: Residence times of trace metals in the SML. There is a section where the authors argue that most of the material in suspension is of Atlantic or European origin except for a few exceptions. Then in order to calculate the residence times of different metals in the SML the authors assume that all metals are present in particles of a certain size except for iron that is in mineral particles ten times higher; and this assumption is for the whole dataset. It is true that if dust is present, its contribution to the rest of the metals measured in this work would be at least 2 orders of magnitude below iron levels (Guieu, Dulac et al. 2010). This supports that Saharan aerosols are not the main source of trace metals. Then why is it suggested that Fe is in thicker particles of "mineral" origin from a different source? Furthermore, there is no relationship between iron levels (high, > 100 ng m<sup>-3</sup>, in 5 samples) and the proximity to the Sahara or the trajectories shown in the supplementary material or the referred episodes of wet deposition. In my opinion, there is not enough evidence to argue that iron is present in particles of a different nature and those are 10 times bigger. I suggest that the authors repeat calculations assuming all the particles have a common origin

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and size and then if they want to keep their original assumption, discuss Fe using two scenarios.

**Authors' Response:** The measurements of aerosol composition during the cruise show a positive correlation between Al and Fe atmospheric concentrations whatever the period and with an enrichment factor for Fe close to 1, meaning a main crustal source for Fe (see attached figure R1). This result is consistent with the literature which show that the Fe deposition in Mediterranean Sea is mainly associated to mineral dust particles whatever the period of year, even during the period when air masses are from European region (Guieu et al., 2010, Desboeufs et al., 2018). Even if a part of iron is anthropogenic and associated to fine particles, this fraction is negligible (in mass) in comparison to iron dust-bearing. So, we added these arguments in the text to explain the choice to use a velocity of 1cm/s for Fe. The text now reads: " During the cruise, Al and Fe atmospheric concentrations were correlated at all the stations and the ratio Fe/Al is typical of a crustal source (Fu et al., in prep.). It is known that the atmospheric iron deposition fluxes are associated to mineral dust particles even during the period when the Saharan dust inputs are very low (Desboeufs et al., 2018; Guieu et al., 2010). On the contrary, no correlation with Al is observed for the other metals, except during FAST1-3.". Also, we realized that we made a mistake in the residence time calculation because we used the aerosol metal flux of the first station (station 1) to estimate the residence time of all stations. We have revised and corrected the calculations and now residence time is calculated using the aerosol flux for each station. Recalculated residence times are of the same order of magnitude than before, however it changed the discussion. For example, now we don't have any significant correlation of residence time to wind speed (see table 3). In the previous version residence time of Co was very well correlated with wind speed, which opened the question on the lack of effect of wind speed on the other metals. Now the relative low wind speed during our campaign ( $9 \pm 4.99$  knots) did not affect the residence time of metals in the SML. It has been indicated the section 3.2.2. - Desboeufs, K., Bon Nguyen, E., Chevallier, S., Triquet, S., and Dulac, F.: Fluxes and sources of nutrient and trace metal

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atmospheric deposition in the northwestern Mediterranean, *Atmos. Chem. Phys.*, 18, 14477-14492, <https://doi.org/10.5194/acp-18-14477-2018>, 2018. - Guieu, C., Loÿe-Pilot, M.-D., Benyahya, L. and Dufour, A.: Spatial variability of atmospheric fluxes of metals (Al, Fe, Cd, Zn and Pb) and phosphorus over the whole Mediterranean from a one-year monitoring experiment: Biogeochemical implications, *Marine Chemistry*, 120(1–4), 164–178, doi:10.1016/j.marchem.2009.02.004, 2010.

**Reviewers' Comment:** The use of high regressions as a cause-effect relationship between variables, specifically the whole discussion about Ni toxicity for bacterioplankton. This needs to be toned down several notches. Although possible, high correlations are indicative of a distribution dependent of common causes and not necessarily of a toxic relationship. If that was the case, salinity would be very toxic for bacterioplankton since the regression coefficient is even higher than that of Ni. Ni concentrations in phytoplankton (I am not familiar with bacterioplankton) are quite high (Twining and Baines 2013) despite their limited physiological relevance without causing deleterious effects. Moreover, in the sampled waters, there is a factor of only two between the highest and the lowest Ni concentrations. It is very unlikely that such a small variation can cause strong toxic effects. I simply do not buy the hypothesis, could be mentioned but only as a hypothesis and I advocate from its removal from abstract and conclusions.

**Authors' Response:** The reviewer is right and we agree that a high correlation between two parameters means a relationship but not necessarily a cause-effect. During the preparation of the manuscript we deeply discussed this point and we concluded that toxicity could be possible although difficult to demonstrate with the available data. Some clues that supported our hypothesis were: 1) the strongly negative correlations between dissolved Ni and microbial abundance; 2) Ni toxicity in the same region was previously suggested although in that case with concentrations 13 times higher than measured during our camping; and 3) although we agree with the reviewer that Ni is not likely to experience redox changes in seawater conditions, the intense UV radiation on the SML can affect the binding capacity of the organic matter and affect to

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its bioavailability and/or potential toxicity. Even so, we are aware that this hypothesis remains speculative and we don't have enough information to demonstrate it. As suggested, we have toned down this conclusion along the manuscript. For example, the last sentence of the abstract (i.e. "Our results suggest a toxic effect of Ni on neuston and microbiology community's abundance of the top meter of the surface waters of the Western Mediterranean Sea") has been replaced by "Our results show a strong negative correlation between the Ni concentration and heterotrophic bacterial abundance in the SML and SSW, but we cannot ascertain whether this correlation reflects a toxicity effect or is the result of some other process." We have also modified the last sentences in the conclusion's section, and now reads: "A strong negative correlation between the Ni concentration and heterotrophic bacterial abundance in the SML and SSW could be suggestive of an inhibiting role of this element on the microbial growth in the top metre of the surface; however, further research is needed to confirm this finding."

Reviewers' Comment: I would also like to see a better explanation about the striking accumulation of certain metals in the SML despite their absence in aerosols (Cd, Mo Pb) even if using bibliographic water column values.

Authors' Response: The accumulation of metals in the SML is controlled mainly by their particle-reactive properties. Thus, Cd and Mo are not enriched in the SML while that Pb is, together with Fe and Cu, concentrated in this layer as result of their binding capacity to particles and organic matter. This has been demonstrated in other regions under the influence of very different sources (e.g. ice or African dust) (Tovar-Sánchez et al. 2019). On the other hand, in addition to aerosols there are other sources that can influence the metal composition of the SML, such as floating material (mainly biological) coming from the water column.

-Tovar-Sánchez, A., González-Ortegón, E. and Duarte, C. M.: Trace metal partitioning in the top meter of the ocean, *Science of The Total Environment*, 652, 907–914, doi:10.1016/j.scitotenv.2018.10.315, 2019.

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Reviewers' Comment: I would also like to see d and aerosol mass data in the final version of the manuscript. Authors' Response: We have included these data in a new figure (Figure S3).

Reviewers' Comment: Comments Page 1 "the total fraction of some reactive metals in the SML (i.e. Cu, Fe, Pb and Zn) showed negative trends with salinity, these trends of concentrations seem to be associate to microbial uptake". Here we have again the problem that a positive or negative high correlation cannot directly be interpreted as a cause-effect relationship. For such statement the authors have first to show that the microbial biomass found in their oligotrophic samples can make a dent in metal concentrations in those waters (from known metal:C ratios). I would find very surprising that the trace element microbial budget is significant when compared to the trace metal phytoplankton budget. Second, why for Cu, Fe, Pb and Zn a negative correlation is indicative of uptake and for Ni is indicative of toxicity? Pb is far more toxic and Ni cellular quotas (at least in phytoplankton, Twining papers) are very high in healthy cells. What are the regression coefficients of those trace elements with respect to salinity?

Authors' Response: In this case, we refer only to the total (unfiltered) fraction of the SML, which include the microbial metal pool (the dissolved fraction of the SML were not correlated either with underlayer water or salinity gradient). Again, the reviewer is right in the fact that conclusion is obtained from linear regressions between parameters. However, in this case the variations of metal concentrations in the T-SML are also positive and significantly correlated with the microbial abundance. We are aware that total fraction includes also lithogenic material, however aerosols metal concentrations did not show any longitudinal trend and no other natural or anthropogenic sources were identified in the region, therefore we think that biological uptake could be reasonable cause. We have clarified this and sentence in page 1 reads: "In contrast, the total fraction of some reactive metals in the SML (i.e. Cu, Fe, Pb and Zn) showed a negative correlation with salinity and a positive correlation with microbial abundance, which might be associated with microbial uptake". In the case of Ni, we found the negative

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correlation with microbial abundance in both compartments, SML and SSW, and in both fractions, dissolved and total. Therefore, toxicity could be a plausible cause. In the case of Pb, there is not a significant trend with salinity. And the correlations with biological abundance is, when significant, positive.

Reviewers' Comment: Page 2 5-10 Questions for the authors: Is the relevance of dust deposition also related to the lack of major riverine discharge? The enrichment at surface is not related to the combination of minimum mix with adjacent seas and strong evaporation (close basin)?

Authors' Response: Of course. The lack of river discharges makes more significant the contribution of aerosol deposition to the metal budget in the water column of the MS. We agree that, in a mass balance, we should consider the fact that evaporation exceed precipitation and river discharges in the MS, generating a hydric deficit that is partially compensated with a limited water exchange with the Atlantic Ocean through the narrow Strait of Gibraltar. However, although many processes contribute to the enrichment of the surface water in some point (e.g. submarine groundwater discharges), we consider the aerosol deposition as the more significant in the top meter of the water column of the MS.

Reviewers' Comment: 13 I would write here may play since most of the following text are considerations and hypotheses.

Authors' Response: Done. The text now reads: "For example, it has been hypothesized that the high Co concentrations in the MS may stimulate "de novo" synthesis of vitamin B12 as Co is the central metal ion in the B12 molecule (Bonnet et al., 2013)".

Reviewers' Comment: 21 I suggest to define the thickness of this SML or at least what the authors consider here (a brief description of the Wurl formula and the parameters it depends upon) since d data are not shown.

Authors' Response: We have included the Wurl formula and described the parameters

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used in the method section 2.2.1.

Reviewers' Comment: 25 The 3 orders of magnitude wide range provided is too much non definition. Are there many different ways to calculate this thickness?

Authors' Response: Generally, the wide range of the thickness of the SML used for different authors is not a calculation issue but rather a sampling issue. The system used for sampling this layer provide you with more or less thickness (e.g. glass plate, rotating drum or screen). In the case of the glass plate sampler used here the thickness is typically 20-150  $\mu\text{m}$  (Cunliffe and Wurl, 2014). - Cunliffe M., Wurl O. (2014). Guide to Best Practices to Study the Ocean's Surface. Plymouth: Occasional Publications of the Marine Biological Association. <https://www.oceanbestpractices.net/handle/11329/261>

Reviewers' Comment: Page 3 1 "Characterized by the dominated abundance of microorganisms" bad grammar. Authors' Response: It has been corrected. Now reads: "Characterized by a high abundance of microorganisms..."

Reviewers' Comment: 3 please remove although. One part of the sentence is not modifying the other 9 influences Authors' Response: Corrected.

Reviewers' Comment: 10 "concentrations of Cu, Fe or Pb in the SML increase by a factor of up to 800, 200 and 150 times compared with the underlying water". Interestingly, this is not the case here. This has to be discussed in detail later on. Authors' Response: Responded below.

Reviewers' Comment: 18 This is likely long enough to be chemically missing word? and biologically missing word? alter the SML and affect the composition and activity of the neuston community Authors' Response: We have changed the sentence. Now reads: "This is likely to be long enough to alter the SML chemically and biologically and affect the composition and activity of the neuston community".

Reviewers' Comment: Page 4 Section 2.1 is quite confusing and the quality of English drops substantially. It has to be revised (grammar and spelling) and modified 14 Is this

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sentence correct and/or complete? It does not make much sense to me. This inlet was developed for sampling both fine and coarse particles, with particles of aerodynamic diameter of about 40  $\mu\text{m}$  Authors' Response: We modified the sentence for clarification and we have revised and corrected the English grammar in the section and along the manuscript. For example, regarding 40  $\mu\text{m}$  diameter we have indicated that: "This inlet was developed to sample particles with an aerodynamic diameter inferior to 40  $\mu\text{m}$  (Rajot et al., 2008)."

Reviewers' Comment: 18 No bibliographic mention to the combination of standard optical and electrical mobility analyzers?

Authors' Response: This combination is the association of two commercial instruments to have a large spectrum of sizes: an OPC for particle size distribution from 0.25 to 32  $\mu\text{m}$  diameter and a SMPS for particle size distribution from 10 nm to 450 nm diameter. We have added the information about these instruments and the text now reads: "The aerosol size distribution from 10 nm to 30  $\mu\text{m}$  was measured by a combination of standard optical and electrical mobility analysers (SMPS, TSI Scanning Mobility Particle Sizer and GRIMM Inc. optical particle counters - OPC, 1.109)."

Reviewers' Comment: 20 a filtration unit 23 all filters / rinsed 23 please rewrite "A sampling strategy was made to avoid the contamination by the cruise smoking" Here add a period and then First 25 the PEGASUS container and the boat's chimney / opposite side of the deck (opposite ship boards?) 28 bad grammar again. Authors' Response: This sections has been corrected and where necessary rewritten.

Reviewers' Comment: Page 5 6 Not all metals measured are presented here. Why Cr and Nd are not included? Authors' Response: Since these two elements (and also Mn) were not analyzed in surface waters and either discussed for aerosol interpretation, they have been removed.

Reviewers' Comment: 7 Why rain data are not commented? Authors' Response: Concentration of metals in rain has been included in section 3.1.

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Reviewers' Comment: 18 the glass plate is not conditioned to the seawater matrix before first collection? I wonder how much metal is adsorbed and extracted from the sample from a plate which surface has been activated after acid cleaning and has only be risen with ultrapure water. Can the authors discard that the first extraction of the day is not lower?

Authors' Response: After rinsed with ultrapure water in the lab on board, the glass plate (and the whole glass plate system) were rinsed in station with seawater several times, and before sample collection the three first dips (SML samples) were discharged. This info has been now indicated in the manuscript and the text in section 2.2.1 now reads: "Once at the station, the glass plate and the whole sampler were rinsed with seawater several times, and the three first dips (SML samples) were discharged".

Reviewers' Comment: 21 what was the result of blank checking? Please describe briefly. Here I also warn that if the blank is run immediately after the ultrapure rinsing, metals could be adsorbed by the plate.

Authors' Response: In all cases blank signals were always lower than 20% of the sample signals for all elements. This has been indicated in the text that now reads: "The sample signal to blank ratio was typically greater than 5:1 for all elements". We really don't know how much metals from ultrapure water are adsorbed to the plate but we assume that it will be equal or less than those absorbed from seawater samples.

Reviewers' Comment: 23 Wurl's formula? /The total. . . . was directly 24 while the. . . . Authors' Response: Wurl's formula has been included. And grammatical errors corrected.

Reviewers' Comment: Page 6 5 why only samples for totals were UV digested? Metal organic ligands and DOM were certainly present in the dissolved samples. Cu and Co analysis in dissolved samples are especially dependent in this digestion step (Rapp, Schlosser et al. 2017).

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Authors' Response: The reviewer is right in the fact that organic ligand and DOM are present in the dissolved fraction. In the case of Rapp et al 2017, the UV digestion has been demonstrated to be necessary for a complete or better determination of Co and Cu using the SeaFAST (i.e. using a particulate chelating resin). In our case, where an organic liquid-liquid extraction using APDC-DDDC was used, the UV radiation is not considered a critical step. However, in the case of the total fraction, and awarded of the high content of MO, we decided to include the UV step to guarantee a full cell breakdown and the complete digestion.

Reviewers' Comment: 23 Microorganisms in the . . . . . were sampled at the same time than. . . . .using a Authors' Response: Corrected

Reviewers' Comment: 25 what does it mean "manually sampled"? I hope not what it literally indicates. Authors' Response: For sampling the seston in the SSW the bottle was dipping directly in the water. To do that and to avoid any organic contamination, hand and arm, were covered with gloves and sleeve protectors.

Reviewers' Comment: 29 please split sentence in two Authors' Response: Done

Reviewers' Comment: Page 7 Sections 2.3.1 and 2.3.2 are almost free of the bibliographic references where the methodologies have been proved for these specific purposes. Example: "value of 26,000  $\mu\text{gC L}^{-1}$  was used for the concentration of dissolved inorganic carbon", where is this value coming from?

Authors' Response: We have included more bibliographic references to support our statements. Primary production rates were calculated by taking into account the mean concentration of dissolved inorganic carbon (DIC) measured during the cruise (26,661  $\mu\text{g/L}$ ). The relevant sentences in section 2.3.2 have been modified accordingly.

References included in this section are: - Harvey G. Microlayer collection from the sea surface> a new method and initial results. *Limnol Ocean* 1966; 11: 608-613. - Cunliffe M, Wurl O. Guide to Best Practices to Study the Ocean's Surface. *Occas Publ Mar Biol*

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Assoc United Kingdom 2014. - Marie D, Partensky F, Jacquet S, Vaultot D. Enumeration and Cell Cycle Analysis of Natural Populations of Marine Picoplankton by Flow Cytometry Using the Nucleic Acid Stain SYBR Green I. *Applied and Environmental Microbiology* 1997; 63:186-193.

Reviewers' Comment: 14 I guess fumes were used before filter use. Not clear with the current sequence. Authors' Response: After the filtration, filters were exposed to HCl fumes in order to remove the non-fixed, inorganic  $^{14}\text{C}$ . The sentence has been clarified accordingly.

Reviewers' Comment: 26 linear least squares regression? Authors' Response: Yes, corrected.

Reviewers' Comment: Page 8 4-5 I find that here the bibliographic revision is too short. There are many more works on the presence of metals in dry aerosols. I would be interested in a very simple study about temporal trends adding studies from the 90s (Roy Chester and several others). In any case the bibliographic search has not been good enough.

Authors' Response: We have included more references in this section. We have added a sentence about the temporal trend to support our observation. The text reads: "However, it has been shown that aerosol concentrations of anthropogenic trace metals (i.e. Pb, Cd and Zn) have decreased remarkably over the last two decades, while crustal metals have not shown any evolution (Heimbürger et al., 2010)."

Reviewers' Comment: Lines 7-10 Here the discussion is very difficult to follow. Authors' Response: It has been rewritten.

Reviewers' Comment: Figure 1 does not include sampling dates and figure S1 is confusing with so much overlapping of curves of similar colours. Then it is difficult to follow this discussion. For me it is like all the trajectories do not show Saharan sources but on those two dates the African input was so high that in those cases particle trajectories

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were “not convenient” and sided for interpretation. Could the authors be clearer about the use of the different information sources? Total mass collected is not provided in the manuscript.

Authors' Response: Date and time of sampling stations are given in Table 1. We think that including dates on the Figure 1 will make the figure less legible. We have indicated the period of sampling in the figure caption. We have included the station name in the Figure S1 for better interpretation. Also, attached is the backward trajectories for the stations 9 and Fast1-4 (Figure R2), and only stations 9 and Fast 1-2 could be influenced from African dust. It has been modified in the manuscript that now reads: “The composition of metal aerosols was mainly influenced by air masses from the North of Europe and Atlantic Ocean (Figure S1), except between June 1st and June 5th (i.e. for the stations St 9 and Fast 1-4) when African air masses were loaded with dust (Figure S1-2).” Total aerosol mass collected is now provided in a new figure (Figure S3). Reviewers' Comment: 9 loaded with? Authors' Response: Yes, corrected.

Reviewers' Comment: Lines 15 to 20 In my opinion this section has to be revised by an English native speaker. Furthermore there are comments about data that are not shown in tables or graphs.

Authors' Response: We have revised and corrected the English grammar in this section and along the manuscript. We have included new figure that refers to cited data.

Reviewers' Comment: 27 trace metals conc of. . . . ., with the exception of Pb, were lower than those measured. . . . . in previous MS studies. “In previous studies” but only one manuscript is cited. I stress that the bibliographic search on trace metals in dry deposition in the Mediterranean area has to be extended and results put in that context before publication

Authors' Response: Unfortunately, up to our knowledge the only data of trace metals in the SML in the MS is from Tovar-Sánchez et al., 2014. Nevertheless, as indicated by the reviewer we have extended the biographic on trace metals in dry deposition in

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the MS.

Reviewers' Comment: Page 9 This discussion is very hard to follow unless ranges supporting arguments are provided in the text. It forces the reader to go back and forth to Table 1 that is actually quite hard to read. Authors' Response: To facilitate the reading we have included ranges to support our statements.

Reviewers' Comment: 7 My question here is how rain affects SML composition and thickness Authors' Response: The atmospheric fluxes of trace metals in the dust wet deposition event are higher than the dry deposition fluxes estimated from aerosol concentrations during the dusty period (Fu et al., in prep). The wet deposition enables to wash-out all the thickness of atmospheric boundary layer and in particular dust which are transported in altitude (Desboeufs et al., 2010). Moreover, the higher concentration of metals in rain depositions is due to the light rain event and low volumes of rain collected during our campaign as consequence of the so-called ‘wash-out’ effect at the onset of rain events (Helmers and Schrems, 1995; Chance et al. 2015). This could explain why during Fast 3 (affected by the dusty rain events) the concentration of some metals in the T-SML were significantly high. We think that the slight rain event did not affect the thickness of the SML. We have included the total trace metals concentrations in the dusty rain to support the potential effect of rain on the SML composition during FAST-3 (P10, L8): " The total trace metal concentrations in the dusty rain collected, ranged from 180 pM for Cd to 343 nM for Fe (Cd: 180 pM , Co: 1380 pM , Cu: 18.1 nM, Fe: 343 nM, Ni: 9.9 nM, Mo: 875 pM, V: 26.9 nM, Zn: 345 nM and Pb: 788 pM)."

- Desboeufs K. , E. Journet, J.-L. Rajot, S. Chevallier, S. Triquet, P. Formenti, and A. Zakou Chemistry of rain events in West Africa: evidence of dust and biogenic influence in convective systems, *Atmos. Chem. Phys.*, 10, 9283-9293, doi:10.5194/acp-10-9283-2010, 2010. - E. Helmers, O. Schrems. Wet deposition of metals to the tropical North and the South Atlantic Ocean *Atmos. Environ.*, 29 (18) (1995), pp. 2474-2484 - R. Chance, et al. Atmospheric trace metal concentrations, solubility and deposition fluxes in remote marine air over the south-east Atlantic Mar. *Chem.*, 177 (Part 1)

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(2015), pp. 45-56

Reviewers' Comment: 8-17 this is a very interesting paragraph. Please discuss the low SML/SSW ratios in the context of the huge ratios referred in the introduction for Cu, Fe and Pb (p 3, 10-11). For Ni, V and Fe the authors should say explicitly that there were no differences between SML and SSW (average close to 1 and standard deviation bigger than the difference). What are the removal processes the authors suggest? Differential dissolution of different metals from the same material? Radiation driven processes? Is taken into account the high efficient mixing in the turbulent 1st meter of the ocean?

Authors' Response: The comparison indicated in the introduction is between the unfiltered fraction of the SML versus the dissolved fraction of the underlayer water. This has been clarified in the introduction section, and now reads: "For example, in regions under the influence of dust events, such as the North Atlantic Ocean or Mediterranean Sea, concentrations of Cu, Fe or Pb in the total pool of the SML are up to 800, 200 and 150 times higher than in the dissolved metal pool of the underlying water (Tovar-Sánchez et al., 2019)". The reviewer is right in the fact that for dissolved V and Fe, the differences of concentration between compartments are not significant when averages are compared, however there were significant differences for some particular stations. For example, for Fe (St6): 13.7 nM (D-SML) vs 2.3 nM (SSW); V (ION-1): 38.2 nM (D-SML) vs 18.7 (SSW). We have modified the text and now reads: "The SML to SSW concentration ratio for V ( $1.2 \pm 0.42$ ) and Fe ( $1.3 \pm 1.5$ ) indicated only slight enrichment in the SML over the underlying water, while the ratio for Mo ( $1.0 \pm 0.1$ ) indicated no difference between layers (Table 1)". Since comparison here is between dissolved fractions, diffusion is likely the main mechanism that provide differences among compartments. Although the mechanisms of dissolution processes for each metal in the SML are not been addressed yet, we believe that photoreaction due to intense UV radiation is the main driver processes. This has been now discussed in the manuscript (section 3.2.1.).

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Reviewers' Comment: 18-20. An efficient mixing should be given by close values (as both watermasses mix efficiently they have the same concentrations) and not simply by high regression. If the slope is close to 1, there is good mixing (line constitutes by identical values, if the slope (not  $r^2$ !!!) is different from 1 that means poor mixing since one of the concentrations is consistently higher than the other and that would mean gradients.

Authors' Response: The reviewer is right. We have replaced the word "mixing" by "transfer", the text now reads: "...indicating an efficient diffusive transfer between these two compartments for these elements." Now, a high regression is indicative of a good transfer rather a good mixing.

Reviewers' Comment: Page 10 Cu and Fe experience redox changes as a function of the solar radiation and Pb has a limited solubility of inorganic forms at pH 8. I do not know whether this explains their distribution but I think it is worth mention it.

Authors' Response: We have included information on different chemical properties of different metals to highlight the complexity in understanding the solubility process that is happening in SML. We have included in the last paragraph of section 3.2.1. the next information: "On the other hand, the complex matrix of the SML and the particular organic and inorganic speciation of each metal studied in the SML will affect their distribution. Thus, for example, Cd and Zn characterized by an oxidation state number of II can vary from very weak to very strong complexation. Lead in oxygenated seawater is partitioned between chloride and carbonate complexes, while Fe and Cu speciation are strongly influenced by pH (Byrne, 2002)."

Reviewers' Comment: 15-16 this statement disentangling metals from particles sizes is very concerning to me. The statement assumes that 1 Fe is included in some particles and the rest of metals in other particles 2 particles including Fe are so much bigger that sink at 10 times faster speed. I think this requires more discussion, if all metals were part of the same particles and no other process was accounted, this would

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underestimate Fe residence time by a factor of 10 and its residence time would be perfectly aligned with those of Cu, Zn, V and Pb. First, previous discussion in this manuscript concluded that most of the aerosols had a European or NA origin. Now the authors consider that Fe has a mineral behaviour far from fine anthropogenic particles. Second, I am not familiar with studies showing that fine particles are low in iron with respect to the rest of the metals in this study, especially those found at the same order of magnitude. If the rest of the metals come from a different thinner material, and some are at concentrations close to the Fe conc in aerosols, then this thinner material is iron free. Third, this sedimentation velocity through the mixed layer is going to be strongly dependent on the energy of the system and a single value for the whole cruise at any location seems a huge source of error to me. Often we have to make simplistic assumptions but I would like that the authors at least make the effort to discuss the consequences of their decisions in terms of uncertainty. How variable was the mixed layer depth during the cruise?

Authors' Response: The measurements of aerosol composition during the cruise show a positive correlation between Al and Fe atmospheric concentrations whatever the period and with an enrichment factor for Fe close to 1, meaning a main crustal source for Fe. This result is consistent with the literature which shows that the Fe deposition in Mediterranean Sea is mainly associated to mineral dust particles whatever the period of year, even during the period when air masses are from European region (Guieu et al., 2010, Desboeufs et al., 2018). Even if a part of iron is anthropogenic and associated to fine particles, this fraction is negligible (in mass) in comparison to iron dust-bearing. So, we added these arguments in the text to explain the choice to use a velocity of 1 cm/s for Fe. The text now reads: " During the cruise, Al and Fe atmospheric concentrations were correlated at all the stations and the ratio Fe/Al is typical of a crustal source (Fu et al., in prep.). It is known that the atmospheric iron deposition fluxes are associated to mineral dust particles even during the period when the Saharan dust inputs are very low (Desboeufs et al., 2018; Guieu et al., 2010). On the contrary, no correlation with Al is observed for the other metals, except during FAST1-3."

C19

- Desboeufs, K., Bon Nguyen, E., Chevaillier, S., Triquet, S., and Dulac, F.: Fluxes and sources of nutrient and trace metal atmospheric deposition in the northwestern Mediterranean, *Atmos. Chem. Phys.*, 18, 14477-14492, <https://doi.org/10.5194/acp-18-14477-2018>, 2018. - Guieu, C., Loÿe-Pilot, M.-D., Benyahya, L. and Dufour, A.: Spatial variability of atmospheric fluxes of metals (Al, Fe, Cd, Zn and Pb) and phosphorus over the whole Mediterranean from a one-year monitoring experiment: Biogeochemical implications, *Marine Chemistry*, 120(1-4), 164-178, doi:10.1016/j.marchem.2009.02.004, 2010.

Reviewers' Comment: 19 I think the shortest residence time in table 3 is 1.2 minutes and not 12. Authors' Response: The reviewer was right. However, residence time has been recalculated as explained before.

Reviewers' Comment: 24-25 I could not find d values in tables. In Wurl's equation d is a function of the sample volume, number of dips and the screen area with the assumption that the presence of surfactants would increase the volume retained per dip and therefore d. It is necessary to have d values if we want to evaluate its impact and variability on residence time calculations. Authors' Response: d values and thickness of the SML have been included in Table 4.

Reviewers' Comment: Page 11 I would not claim that different behaviours are caused by different reactivities to natural ligands. Of the metals targeted in this study, only Zn has a weak affinity for natural organics (not much is known about V affinity for natural organics). Cu is the clear example of strong affinity to ligands and even is known that this affinity is higher than that for biological membranes (González-Dávila, Santana-Casiano et al. 2000). Here the elephant in the room is photochemical processes.

Authors' Response: Unfortunately, we don't know yet what process is conditioning the behavior and distribution of each metal in the SML. By sure, it is not only one but the combination of many (e.g. solar radiation, wind speed, OM contents, neuston composition, etc.) that affect differently each metal according to its reactivity and redox

C20

potentials. In this sense we think that the reviewer comments have significantly helped to extend this discussion. We have extended in the manuscript the potential role of photochemistry in the SML.

Reviewers' Comment: 5 can be known how is  $d$  related to wind force?. No consideration of photoreactions?

Authors' Response: Yes, wind speed is directly related with the thickness of the SML that in turn it affects the number of dips needed to collect the SML sample. For that reason, wind speed was included as one parameter in the statistical test (Table 4). Since not significant correlation was found between metals concentration and wind forces we have removed the discussion about the effect of wind speed and TSML Co concentration. The text now reads: "Wind speed seems not to have affected the residence time of any metal in the SML (Table 3), which is probably due to the low speed registered during our campaign ( $9 \pm 4.99$  knots) (Table 1).

Reviewers' Comment: 16 again it is said of other regions but only one example is provided. Rewrite for this specific case or bring more examples.

Authors' Response: We have included more references to support this statement. Additional references included are: - Engel A, Galgani L. The organic sea-surface microlayer in the upwelling region off the coast of Peru and potential implications for air-sea exchange processes. *Biogeosciences* 2016; 13: 989-1007. - Agogue H, Casamayor E.O., Bourrain M, Obernosterer I, Joux F, Herndl G.J., Lebaron P. A survey on bacteria inhabiting the sea surface microlayer of coastal ecosystems. *FEMS Microbiol Ecol* 2005; 54: 269-280. - Joux F, Agogue H, Obernosterer I, Dupuy C, Reinthaler T, Herndl G.J., Lebaron P. Microbial community structure in the sea surface microlayer at two contrasting sites in the northwestern Mediterranean Sea. *Aquat Microb Ecol* 2006; 42: 91-104.

Reviewers' Comment: 21 "In general, and with the exception of phytoplankton middle and CBLsmall, microbial abundance was higher in the SML than in the SSW with abun-

C21

dances ranging from 1 to 6 times higher for bacteria and CBL-middle-large, respectively (Table 1)". In Table I the groups with a higher concentration in the SML are autotrophs (phyto and cyanobacteria). However, the extremely low Chl-a concentrations in the SSW (low even for oligotrophic waters, consistently below  $0.1 \mu\text{g l}^{-1}$ , actually they should revise their numbers, I only saw numbers that low in the eastern mediterranean) point to a lack of viable autotrophs in the SSW. And here it is difficult to point to UV effects since the SML should receive even more radiation. It is a real pain that there are no Chl-a measurements in the SML to infer whether the higher cellular content was constituted by viable cells. It is also shocking the lack of correlation of Chl-a with any of the biological variables.

Authors' Response: The low Chl-a values were not anomalous. For comparison one can look at the Prosopé (summer/fall) and Boum (summer) cruises: in both cases during most of the longitudinal transect (including the central Med Sea and a good section of the Western Med Sea) surface Chl-a values were below  $0.1 \mu\text{g/L}$  (Crombet et al. 2011 *Biogeosciences*, 8, 459–475). In fact, the Chl-a concentrations were actually very typical of expected ones from satellite observations climatology. The period of the cruise was chosen to be a compromise between very low Chl-a concentration and high probability of dust deposition. According to Chl-a concentration over the whole Mediterranean Sea, our expedition encountered a classical situation regarding Chl-a (i.e. Bosc et al., 2004) (Figure R3). (this is fully developed in the introduction/strategy paper by Guieu et al. 2019). It is much lower in the eastern Mediterranean that can be qualified as ultraoligotrophic with concentrations  $< 0.03 \mu\text{g.l}^{-1}$ . The suggestion that there were 'no viable autotrophs' in surface waters does not seem correct, because the microcosm dust addition experiments (conducted with water from ca. 5 m) showed a response of the phytoplankton community to the nutrients released from the dust. This response was particularly noticeable in the ION and FAST long-duration stations, but existed also in the G treatment at TYR. We have focused the discussion in the most representative microbial community in the SML (i.e. Bacteria; High nucleic acid-content bacteria: HNA; Low nucleic acid-content bacteria: LNA; pico-phytoplankton). We have

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removed the different phytoplankton groups from the text since discussion about their abundance is speculative with the existing data. Also, we have included a paragraph explaining the microbial abundance differences between SSW and SML. The paragraph reads: “Bacterial abundances did not differ significantly between SML and SSW (Table 2). The only slight bacterial enrichment was found after dust input due to an increase in the bacterial cells in the SML, which quickly reverted to the abundances found before the dust input within 48 hours. Phytoplankton was only slightly, but significantly (t-test,  $p=0.002$ ,  $n=12$ ) enriched in the SML with an average enrichment of 1.5 compared to the SSW.”

- Crombet Y., K. Leblanc, B. Que ÌAguiner, T. Moutin, P. Rimmelin, J. Ras, H. Claustre, N. Leblond, L. Oriol, and M. Pujo-Pay. Deep silicon maxima in the stratified oligotrophic Mediterranean Sea. *Biogeosciences*, 8, 459–475, 2011. - Bosc, E., Bricaud, A., & Antoine, D. (2004) Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations, *Global Biogeochemical Cycles*, 18, GB1005, doi:10.1029/2003GB002034. - Guieu C., D’Ortenzio F., Dulac F., Taillandier V., Doglioli A., Petrenko A., Barrillon S., Mallet M., Nabat P., Desboeufs K., Process studies at the air-sea interface after atmospheric deposition in the Mediterranean Sea: objectives and strategy of the PEACETIME oceanographic campaign (May-June 2017), in prep, this issue, 2019

Reviewers’ Comment: 23 rewrite in English please Authors’ Response: done.

Reviewers’ Comment: Page 12 1-2 It makes sense but I would use could instead of would, it is all speculative. I really doubt that assimilation and storage from such a low biomass could explain trace element trends

Authors’ Response: We have toned down this statement. Now it reads: “Bacteria could efficiently assimilate the fraction of Cu, Fe and Zn available, favouring a decrease in the D-SML fraction (Table 1-2)”.

Reviewers’ Comment: 5 Revise English. It is very surprising that TEP concentrations

C23

(of biological origin) could increase after a dust deposition, they should remain or decrease by scavenging. I would tone down this sentence. First it is based on a single value and second it is not higher than Station 9 Authors’ Response: The reviewer is right. We have removed that sentence.

Reviewers’ Comment: 6 “we therefore. . . .” Because there are no correlations between metals and TEP the consequence is metal assimilation by microbes explain longer residence times? I do not follow the cause-effect relation here. Please include here known Cu, Zn and Fe cellular quotas to justify or discard assimilation (Twining papers).

Authors’ Response: We agree with the reviewer. Since the metal assimilation by microbes could be feasible but we cannot demonstrate it, we have modified the sentence as follow: “Metal assimilation by microbial communities could explain the higher residence time of Cu and Zn (in the order of hours) in the SML, although information about the metal content in seston would be necessary to corroborate this hypothesis.”

Reviewers’ Comment: 10 and here appears the elephant in the room. It must be taken into account the complexity of photochemical reactions (reducing Fe and Cu) but also the bleaching effect on DOM and ligands. Authors’ Response: Yes, we agree. Discussion about this issue has been included in section 3.2.1

Reviewers’ Comment: 11 That Ni is strongly anticorrelated to bacterioplankton is indicative of a relation but not necessarily direct. It could be (as for other metals) that is taken up and it is not toxic; as a possible result the higher the bacterial density, the lower the Ni concentration. Authors’ Response: We tried to argument this in previous comments. In any case, we have toned down this hypothesis along the ms.

Reviewers’ Comment: Figure 3. Are those least square linear regressions? Authors’ Response: Yes, they are. It has been indicated in the caption of the Figure 3.

Reviewers’ Comment: 17-19 please give data ( $r^2$ ) 23 “close correlated” closely. Au-

C24

thors' Response: Done

Reviewers' Comment: Page 13. There is a lot of discussion about possible mix Atlantic and MS waters but no actual bibliographic search on average values in both waters that could justify that some metals could be enhanced by mixing and others not. Please, look for such data.

Authors' Response: The surface distribution of metals in the western Mediterranean Sea is known to be impacted by the Atlantic inflow water (e.g. Morley et al. 1997; Gómez 2003 and references therein). However, this discussion has been always focused on the surface layer considering the layer below 10 m. Although, undoubtedly it is considered relevant in the global surface distribution of metals, we believe that in the study of the SML other factors such as aerosol deposition or chemical and biochemical issues are more relevant.

- Morley N.H., Burton J.D., Tankere S.P.C. and Martin J-M. 1997. Distribution and behaviour of some dissolved trace metals in the western Mediterranean Sea. *Deep-Sea Research II*, Vol. 44, No. 34, pp. 675-691. - Gómez F. 2003. The role of the exchanges through the Strait of Gibraltar on the budget of elements in the Western Mediterranean Sea: consequences of human-induced modifications. *Marine Pollution Bulletin* 46 (2003) 685–694

Reviewers' Comment: 16" Indeed, UV radiations in this surface layer are highly intense and can acts as a biochemical microreactor where many transformations and photochemical reaction occurs" rewrite after grammar checking. I find that claiming that photoreactions could explain this bioaccumulation is really far fetched. Specially for a metal that has no different redox states in oxygenated seawater

Authors' Response: Considering this and previous reviewer's comments we have rewritten this part. Now, we discuss (as follow) the potential role of UV radiation on the dissolution (and bioavailability) of Ni: "The toxicity to phytoplankton of divalent, cationic trace metals, such as Ni or Cu, is probably controlled by its free metal ion

C25

concentration (Donat et al., 1994). Although the Ni interactions with dissolved organic matter have not been studied well in seawater, they are thought to occur partly as stable organic complexes and with slow dissociation rates (Wen et al., 2011). However, intense UV radiation can alter the concentration, structure, reactivity and metal binding capacity of the organic matter, thus increasing the proportion of free metal ions and their bioavailability and/or potential toxicity (Cheloni and Slaveykova, 2018)."

Reviewers' Comment: Page 14 "It appears that Nickel-dependent toxicity involving ROS may be likely mechanism of oxidative stress in marine microbial organism of the surface ocean" check grammar but better discard here

Authors' Response: We have toned down this statement and delete the last sentences. The paragraph finish as follow: "It appears that nickel-dependent toxicity involving ROS could be a mechanism of oxidative stress in microbial organisms of the surface of oceans. While the effect of Ni on microalgae has been studied with laboratory cultures (Brix et al., 2017; Macomber and Hausinger, 2011, 2016), its potential toxic role in the surface of oceans has not yet been investigated."

Reviewers' Comment: Conclusions Is Co not affected by chemical and biological processes? That is very surprising due to its important requirement Authors' Response: We have modified this sentence. Now reads: "While some metals entering the SML (e.g. Cd, Co, Ni and V) show efficient diffusive mixing from the SML to the SSW, more reactive metals such as Cu, Fe, Pb and Zn seem to exhibit a slower diffusion".

Reviewers' Comment: Figure 1. This is a good figure but I do not understand why has been sent vertical. I guess for the publication will be required a reduction in size, shift to horizontal and increase of the font size. Authors' Response: We have changed the format and now is in horizontal.

Reviewers' Comment: Figure 2. I guess DN<sub>i</sub> refers to DN<sub>i</sub> in the SSW. Please reduce size. I am not sure this relationship deserves a whole figure. 1 the regression coefficients are in the tables. Second, the supposed bacterioplankton control by Ni toxicity

C26

is a nice hypothesis but data do not prove such dependence.

Authors' Response: DNI refers to both, SML and SSW: now, it is clarified in the caption. We think that this significant correlation deserves to be plotted since it is found in both, SML and SSW. Although we agree with the reviewer that the correlation does not prove the Ni toxicity it is the base for our argumentations.

Reviewers' Comment: Tables are quite difficult to read and I wonder if these will be legible in the final version of the manuscript. In any case all provide useful information and I would not simply remove data from them. Authors' Response: We have splitted the Table 1 in two for easier reading.

Reviewers' Comment: Table 3. Station not satation Authors' Response: Done

Reviewers' Comment: Figure S3. Wrong caption.

Authors' Response: The reviewer is right. Figure S3 (now Figure S4) has been changed with rainfall rates from the radar European composite product that are geo-referenced allowing to plot the position of the FAST station. The figure cation has been changed consequently and now reads: "Accumulated rainfall during the night between June 3rd and 4th 2017 (00h00 – UTC) and position of R/V at the Fast Station. The rainfall rates are estimated from the radar European composite products provided by the Odyssey system." We really thank to the reviewer for all these comments and the many errors detected.

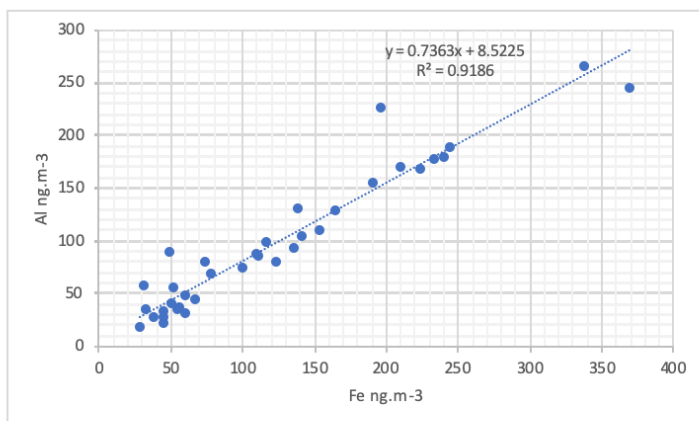
Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2019-290/bg-2019-290-AC1-supplement.pdf>

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-290>, 2019.

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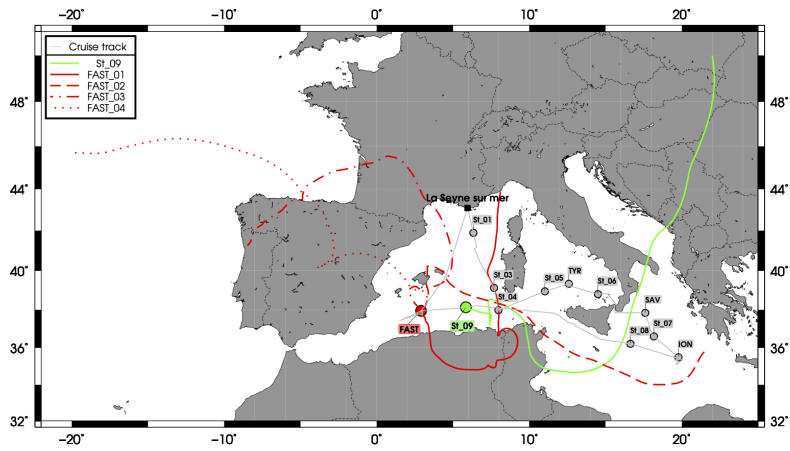
**Figure R1.** Al and Fe atmospheric mass concentrations in all stations along Pacetime cruise (Fu et al. in preparation).



**Fig. 1.**

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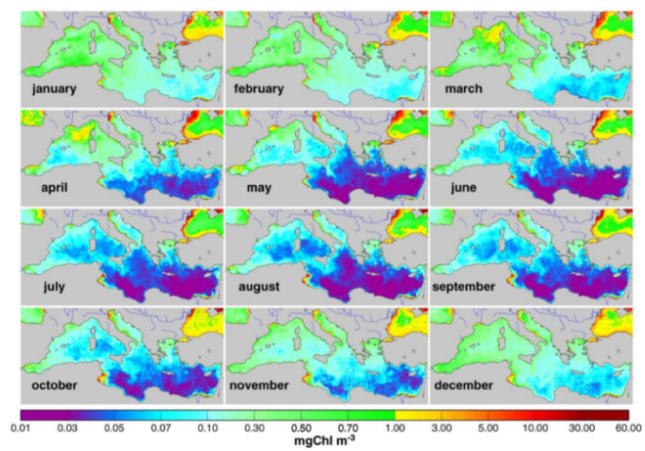
**Figure R2.** backward trajectories for the stations 9 and Fast1-4



**Fig. 2.**

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**Figure R3.** Monthly averaged chlorophyll maps derived from SeaWiFS data for the year 1999. Source Bosc. et al. 2004



**Fig. 3.**

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