Enrichment of particulate phosphorus in a sea-surface microlayer over the Eastern Equatorial Pacific Ocean

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The significant enrichment of chemical composition associated with enhanced bioactivities and an increased number of bio-particles in the sea-surface microlayer (SML) were observed in the high-nutrient, low-chlorophyll (HNLC) eastern equatorial Pacific Ocean (0°N, 95.5°W) during the EqPOS cruise in 2012. The particulate phosphorus and iron were enriched by factors of 72 and 11, respectively, in the SML samples in comparison with subsurface water (SSW) samples. Individual particle chemical analysis by SEM/EDX also showed higher phosphorus levels in most of the analyzed SML particles than in the SSW particles. These observations demonstrated a distinct biogeochemical enhancement in the SML in response to an external perturbation, most likely iron depositions into the HNLC ocean SML.

Keywords: sea-surface microlayer, biogeochemical enrichment, particulate phosphorus, high-nutrient low-chlorophyll ocean

INTRODUCTION

The sea-surface microlayer (SML) is the thin interfacial layer between atmosphere and ocean, and it is distinguished by its differences in biological and physiochemical properties in comparison with subsurface water (SSW) (Cunliffe et al., 2013). SML layer varied significantly from 6 to 1000 µm as a result of different collection devices/methods and meteorological conditions (Cunliffe et al., 2013), such as wave breaking and wind speed. Because ocean surface covers 70% of the earth, SML has been recognized to have significant roles in air-sea exchange of heat, gases, liquid, and solids (Cunliffe et al., 2013; Liss and Duce, 1997), as well as in biogeochemical process for materials synthesis, transformation and cycling (Wheeler, 1975).

Well-known for its enrichment property, SML can also influence chemical distribution and speciation above (in the atmosphere) and underneath water column (sub surface water: SSW) (Hardy, 1982). Reported enrichment factor defined as the ratio of concentration on SML to SSW (Liss and Duce, 1997) of materials varied depending on their species and surface-active affinity, generally approximately 1 to 3 for dissolved organic carbons and nitrogens and inorganic nutrients (Gladyshev, 2002; Liss and Duce, 1997). Higher enrichments were observed for particulate matter, such as particulate organic carbons and particulate trace metals (Liss and Duce, 1997; Hardy, 1982) with enrichment factors of 3–38 and 2–100, respectively. Recently, the abundances of bacteria (Bacterioneuston) and phytoplankton (Phytoneuston) in SML were also reported (Cunliffe et al., 2013), and structures of the microbial community were found to be different from those below surface water because the prevailing meteorological conditions permit a distinctive population at the interface (Stolle et al., 2011).

The SML can be readily influenced by biological processes in water columns and/or meteorological conditions in the atmosphere. However, it is also possible that the biological processes in the SML itself can be affected by a slight external nutrient input, such as a deposition of atmospheric aerosols, because the SML is a thin layer with a markedly small volume. It is worth considering the occurrence of such a mutual biogeochemical link and its variation within the SML. Here, we attempted to clarify the biogeochemical processes occurring within the SML, especially in a region of calm sea conditions, i.e., the equatorial Pacific Ocean, a typical oceanic region displaying specific features of high-nutrient, low-chlorophyll conditions (Kaupp et al., 2011) and an equatorial upwelling zone (3°N–3°S, 90–140°W; Pennington et al., 2006).
**MATERIALS AND METHODS**

Observations were conducted on board the R/V Hakuho Maru from 29 January to 19 February 2012 as part of the Equatorial Pacific Ocean and Stratospheric/Tropospheric Atmosphere Study (EqPOS) campaign. This campaign aimed to better understand the biogeochemical linkages between ocean and atmosphere in the eastern equatorial Pacific (EEP) in which open-ocean upwelling and HNLC conditions have been reported (Kaupp et al., 2011). Phosphorus (P); the interested element in this study is one of the essential elements for marine organisms. The major sources of marine nutrients are river-derived materials and atmospheric deposition, with the latter becoming an important source where the former contribution is minor (Paytan and McLaughlin, 2007) away from rivers and coastal regions. In addition, the nourished water in the research area also brings nutrients up to the ocean surface by the ocean-upwelling process.

SML samples were collected with a glass plate (SML-GP) and a Polymethyl Methacrylate (PMMA) rotating drum (SML-D) in comparison with sub-surface water (SSW, sampled at a depth of 1.5 m by sinking an empty cleaned bottle with narrow mouth with a weight) under calm sea surface conditions on a small boat with a distance approximately 500 m upstream and upwind from R/V Hakuho Maru. These two SML sampling methods are based on a principle that about 20–150 m of surface seawater adhere to the samplers’ surface through the different viscous retention (Liss and Duce, 1997); further details of SML sampling methods are shown in Supplementary Fig. S1). Samples collected by SML-GP and SML-D are regarded as SML seawater and are used for the calculation of the SML enrichment. In order to compare among the results, the term of surface concentration is applied (this term expressed as the subtraction of the concentration in SML and SSW multiplied by its thickness of collection (σ) which is different between sampling techniques and is calculated by dividing the obtained sampling volume by the total collected surface area; Liss and Duce, 1997). Of these two methods, concentration of total particulate phosphorus showed the most comparable trends, while the SML-D samples (obtained more sample volume efficiently) are henceforth referred to as the SML samples in this study. On shipboard, surface water (SUR; approximate depth 0.3 m) collection by bucket sampling was performed along with CTD rosette seawater sampling with Niskin bottles at depths of 5 m, 10 m, 50 m, 100 m and 200 m.

Seawater samples filtered with pre-combusted (550°C; 5 hours) Whatman GF/F filters (pore size: 0.7 µm) were analyzed for total particulate phosphorus (TPP), and filtrate samples were stored in acid-cleaned polypropylene bottles and frozen at −20°C until the analysis of total dissolved phosphorus (TDP) and soluble reactive phosphorus (SRP). SRP was determined by modified magnesium-induced co-precipitation (Kar1 and Tien, 1992) prior to concentration and analyzed with the molybdenum blue colorimetric method (Strickland and Parsons, 1972). Filters used for the TDP determination were pre-oxidized with boric-persulfate oxidation solution (Liu et al., 2010) prior to pre-concentration. With 5-fold pre-concentration, the detection limit of the analysis was 4.0 ± 1.4 nM determined as three times the standard deviation of the lowest molybdenum blue standard. For the total particulate phosphorus (TDP) analysis, filter samples were sectioned and ashed overnight at 550°C, and residuals were later dissolved in 1 mL of 1 N HCl and diluted to 20 mL with Milli-Q water and shaken well for 30 mins (Chen et al., 1985, 2006). Then, the solutions were filtered, pre-concentrated and analyzed by the molybdenum blue colorimetric method (Strickland and Parsons, 1972). The detection limit of the TDP analysis was 2 ± 0.7 nM with 10-fold pre-concentration. Particulate inorganic phosphorus (PIP) or the extractable inorganic phosphate was also measured after the filter extraction with 1 N HCl without ashing process.

Seawater samples filtered with Nuclepore® filters (pore size: 0.4 µm) were obtained and analyzed for particulate elemental composition by an energy dispersive X-ray fluorescence spectrophotometer: ED-XRF (MXF-01, Kimoto Electric Co., Japan) with operating conditions of accelerating voltage 20 kV, beam current 2 mA and collection time of 500 sec/sample (Iwamoto et al., 2009). The detection limit for particulate Al, Si, S, Ca, and Fe were 38, 12, 10, 8, and 2 nM, respectively (equivalent to Al, Si, S, Ca, and Fe; 1030, 330, 260, 300, and 84 ng L⁻¹ for a filtration volume of 2 L).

To compare the datasets from different stations with different absolute concentrations, observed elemental concentrations were also described as an enrichment factor (EF), which expresses as the ratio of the concentration in SML relative to that in SSW:

\[
EF_{x} = \frac{\left[ X \right]_{\text{sml}}}{\left[ X \right]_{\text{ssw}}}\]

where X represents a chemical composition of interest (Liss and Duce, 1997). In addition, sectioned filters (approximately 25 mm²) for SML and SSW sample from station 1 (0°N, 95.5°W) were mounted on carbon stubs and vacuum-coated with carbon (Elzerman et al., 1979) to perform a single-particle morphological and elemental analysis by a field emission scanning electron microscope coupled with energy-dispersive spectroscopy with light-elemental analysis (SEM/EDX, S-4800II, Hitachi Co., Japan) with operational accelerating voltage and beam current of 15 kV and 10 µA with a collection time of 60
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sec, repeated (n = 3–5) for each particle. Particles of the average diameters (D) from 0.4 to 100 µm were size-separated and binned by their diameters, morphology and composition, with cut-off sizes at 2, 5, 10, 20, 30, 40, and 50 µm. Only particles producing elemental signals with a signal-to-noise ratio (S/N) > 3 were used in further analyses. In addition, the intensities of the X-ray depended on the particle’s surface shape and the respective element’s concentration at the shooting point, and the sum of the analyzed elements (O, Na, Mg, Al, Si, P, S, Cl, K, Ca, Fe, Cu, Zn and Ti) was rescaled to obtain a constant sum of 100% (Schleicher et al., 2012). In total, 66 and 45 particles were analyzed with EDX for the SML and SSW samples, respectively.
RESULTS

Concentrations of SRP

Concentrations of SRP in seawater samples collected from SML, SUR and SSW were relatively similar in value and showed neither clear SML depletion nor repletion (as presented in Supplementary Table S1). Higher enrichment factors were generally observed in particulate components for all stations, and a much higher enrichment was observed for TPP and pFe at station 1. Usually, the TPP composed approximately 10% of total phosphorus in the open ocean (Karl and Tien, 1992). However, at station 1 (0°N, 95°E), the TPP concentration in the SML (1.256 nM) was remarkably higher than that in the SSW (18.0 nM), resulting in the highest observed enrichment factor (EF TPP = 72). In addition, this observation was found in concurrence with the increases of pFe (EF pFe = 11) and the other elements such as silicon (EF pSi = 6.1) and sulfur (EF pS = 6.3).

Because the two different SML sampling techniques showed exceptionally high enrichments of TPP and other particulate trace elements only in the SML samples from station 1, suspended particulate matter from SML and SSW at station 1 were examined intensively using a SEM/EDX approach. The numbers of particle (from 0.4–100 µm size) were counted on the known area of the SEM images and the average particle concentration was estimated by dividing the number by the total volume of filtered seawater. The average total particle concentrations in SML and SSW (Fig. 1a) were 3,200 and 3,100 per mL, respectively.

Although the particle numbers were similar between them, the particle size distributions were clearly different. While most of the SSW particles (~79%) were present in the smallest size fraction (0.4–2 µm) among the size ranges observed by current SEM, SML particles were predominantly present in larger size fractions, particularly in the size fractions of 20–30, 5–10, and 2–5 µm, which accounted for 30%, 24% and 19% of the total number of particles, respectively. The volume concentrations in total size fraction was 9.130 ppbv (or x10³ µm³ mL⁻¹) for SML particles or 4-fold larger than that of SSW (1.840 ppbv), which is due to the abundance of large particles in the SML (D > 20 µm) (Fig. 1b).

From particle morphology and elemental composition, the particles collected at St. 1 were categorized into 38 particle types and then grouped into 5 simplified particle groups: diatoms (16 types), dinoflagellates (2 types), silicoflagellates (1 type), other microorganisms (10 types) and other particles (9 types) such as inorganic particles; example micrographs are shown in Supplementary Fig. S2. In the SSW (Fig. 1c-2), the most abundant particles (D < 2 µm) were mixtures of the other-particle and other-microorganisms groups, whereas the particles in the SML (Figs. 1a-1 and 1c-1) were mostly composed of diatoms and other-microorganisms. Interestingly, diatom particles significantly dominated the 2–10 µm fraction of the SML sample. The increase of diatom particles was also observed in the size fraction of 20–40 µm, and the total number of diatom particles (0.4–100 µm) also increased from 290 particles mL⁻¹ in SSW to 1,900 particles mL⁻¹ in SML. Moreover, other-microorganism particles also increased in number and proportion, especially in the particle fractions of 6–10 and 30–40 µm, with the latter being the particle type most prominently observed in the microscopic views during the SEM observation (Fig. 1b-1). Typically, TPP is composed of living and dead plankton, precipitates of phosphorus mineral, phosphorus-adsorbed particulates and amorphous phosphorus phase, which undergoes continuous transformation into dissolved inorganic and organic phosphorus through plankton assimilation and excretion (Paytan and McLaughlin, 2007). The observed tremendous increase of TPP in the SML, which was identified to be caused by the higher number of plankton and other-microorganisms particles, cannot be explained by the ordinary chemical enrichment processes such as bubble floatation, water-mixing and the atmospheric deposition (Liss and Duce, 1997). It should also be noted that Fe-containing particles were observed and were classified in the other-particle group under inorganic-like particles in both SML and SSW samples, but the SML particles had much higher %Fe, as shown in Supplementary Table S2.

The elemental composition

The elemental composition analyzed by the SEM/EDX is presented as the relative percentage of element composition and is reported in Table S2. The SML particles exhibited higher %P content, in which 77% of the total SML particles analyzed were P-detected particles, while only 11% of the SSW particles contained P. The average %P in the SML particles, expressed as a relative weight percentage of the sum of the analyzed elements, was also much higher (4.1%) than in the SSW particles (1.8%) for both the overall average and the same particle types. For comparing the results of the single-particle analysis and the bulk chemical analysis, the single particle SEM/EDX data were used to estimate the amount of phosphorus contained in the particles with the following simple conversion:

\[
\text{TPP}_{\text{SEM/EDX}} = \text{total particle mass concentration} \times \frac{\%P_{\text{avg}}}{100} \times \text{fraction } P_{\text{detected}}
\]

where %P avg is the average %P in a particle sample (Table S2, number in brackets) and fraction P-detected is the fraction of P-detected particles (0.77 and 0.11, for SML and SSW). By applying the volume/mass particle density conversion of 2.2 mg mL⁻¹ (Pak, 1973) to the volume con-
Fig. 2. (a) Vertical profiles of chlorophyll-a (Chl-a), particulate phosphorus (TPP), particulate Fe (pFe), and soluble reactive phosphorus (SRP) observed at station 1 (0°N, 95.5°W) and the station 8 (0°N, 130°W). A large enrichment of TPP and pFe was observed at station 1, and the profiles suggest that there was atmospheric deposition to the surface ocean during the collection. The open markers represent measurements in the SML. (b) Micrographs showing the SML (1–2) and SSW (3–4) particles at station 1 (left panels) and station 8 (right panels).
concentration mentioned above, the total SML and SSW mass concentrations were calculated as 24 and 6 µg L\(^{-1}\), and the TPP\(_{\text{SEM/EDX}}\) values were estimated to be 0.75 and 0.01 µg L\(^{-1}\), respectively. Applying the above TPP\(_{\text{SEM/EDX}}\) conversion, the resultant EF\(_{\text{TPP}}\) was 75. This number was of the same magnitude as the observed EF\(_{\text{TPP}}\) by two different sampling methods (SML-D = 72 and EF-GP = 33), confirming that the observed large enrichment of particulate phosphorus in the SML is not an artifact but a natural occurrence. It should be noted again that such a large enrichment of TPP was not observed in the SML samples collected at the other four stations.

**DISCUSSION**

**Potential cause of the particulate enrichment in the SML at station 1**

Particulate matter is often enriched in the SML, particularly organic particles, as they are readily stabilized at the air-sea surface through surface-tension forces (Liss and Duce, 1997); however, the degree of particle enrichment varies based on several factors: meteorological conditions, the particles’ physicochemical properties (water dissolution, bubble scavenging process; Hardy, 1982), compound surface active properties and atmospheric deposition (Liss and Duce, 1997). In addition, the biological activities in water columns can also affect the chemical partitioning process and the enrichment of particles in the SML (Wurl et al., 2011). At station 1, the increased phosphorus content (%P\(_{\text{avg}}\) = 4.1) was seen not only in individual SML particles but also in the bio-particles (diatom and other microorganisms) in the SML. The observed TPP enrichment in the SML through the amplification of both bio-particles and the internal P-content in each particle cannot be fully explained by the ordinary natural enrichment process.

In addition to the TPP, we also observed the highest concentration of pFe in the SML. The observed vertical profiles (Fig. 2a) show that such concentrations of TPP and pFe were found only in the surface water, suggesting the contribution of the atmospheric deposition of additional nutrients to the surface ocean prior to the sampling. Because this observation was conducted in the HNLC region in which a meso-scale iron fertilization experiment had increased biological responses (Coale et al., 1996) and was also confirmed by an on-board Fe fertilization incubation experiment in surface seawater during the EQPOS observation (Takeda, 2012; personal communication), such aeolian deposition may supply lacking micronutrients; in this context, supplying pFe to the SML, triggering the plankton blooming-like condition in the SML in addition to the typical SML enhancement, resulting in the observed increases in the particulate phosphorus and biological particles in the SML. The microscopic observations (Fig. 2b) also clearly demonstrated the differences in the particle composition in the SML sample at station 1 with the SSW and the samples from station 8 as an example of normal conditions in this region. The aeolian Fe deposition likely stimulated additional primary production within the SML and resulted in formation of more biomass in the SML under the HNLC condition. Although the upwelling water is the most significant iron source for surface ocean in this region (Kaupp et al., 2011), the observed vertical profiles of TPP and pFe and the lack of their enhancement in sub-surface seawater suggested that the observed enrichment in the SML was caused by natural atmospheric deposition. Such a biogeochemical response in the SML should be able to continue for a short time and would be terminated when the SML was disintegrated by wind and wave action or when the deposited atmospheric matter migrated into sub-surface water by sinking or diffusion.

The observed biogeochemical enrichment in the SML emphasizes that SML is not a simple concentrated thin layer in which various chemical and biological substances are enriched but is instead a unique realm that provides an anomalous biogeochemical environment and promotes unique biogeochemical reactions and creating biological structures. Based on the current results showing that a slight amount of additional iron input enabled a dramatic increase in SML biological production, SML is a biogeochemical environment that can be highly sensitive to external perturbation. These dynamic biogeochemical processes in the SML are also expected to influence various physical processes that take place through the SML, including air-sea exchanges of heat, gases, liquids and solids. Because the SML is a small reservoir of material due to its thinness, such biogeochemical responses seem to be not only anomalous but also rapid, indicating its variable nature in time and space.

**CONCLUSION**

The remarkable increase of TPP (1.29 µM) to the same level of SRP (0.84 µM) in the SML and the higher enrichment factor of TPP to 72 were observed during the EQPOS cruise at the eastern Pacific Ocean (0°N, 95.5°W). This finding was concurrent with a dramatic increase of biological-particles, especially diatoms, in which numbers rose to 1,900 particles mL\(^{-1}\) in SML, 7 times higher than that of SSW. Further investigation of individual particles by SEM/EDX demonstrated a higher phosphorus content (%P\(_{\text{avg}}\) = 4.1) in most of the analyzed SML particles (77%), while only diatoms and other-microorganisms particle types of the analyzed SSW particles (11%) contained phosphorus content (%P\(_{\text{avg}}\) = 1.8).

In addition to the elevated particulate phosphorus, particulate Fe concentration was also highest in the SML.
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REFERENCES


(81 nM; EFpFe = 11), and the vertical profiles of TPP and pFe, with peaks in the SML, were noted (Fig. 2). Thus, the most probable explanation for this circumstance was that it is the result of biological responses to the external atmospheric deposition of scarce micronutrients to the SML.

Although SML is quite a small material reservoir, its biogeochemical dynamics and properties are unique. The observed biogeochemical enrichment emphasizes its rapid response to external perturbation and its significant role in microbe-mediated materials synthesis and transformation of chemical compositions and their cycling, further indicating that a slight amount of additional iron input enabled the dramatic increase in SML biological production. This possibly occasional but unique finding of the biogeochemical enrichment in the SML addresses its significance in biogeochemical and physicochemical processes in which SML is involved. Further investigation of the behaviors and mechanisms of materials transformation and retention time in SML will be valuable to clarify the important interface role of the SML in the biogeochemical cycles between atmosphere and ocean.


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