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PIP/PP ratio as an indicator of phytoplankton activities and origin of particulate matter in Harima-Nada, the Seto Inland Sea, Japan

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Abstract : Chemical and elemental components of surface particulate matters were investigated during 4 seasons all over the Harima-Nada, the eastern part of the Seto Inland Sea, Japan. We measured concentration of particulate organic carbon (POC), particulate organic nitrogen (PON), and chlorophyll *a* (Chl.*a*). We also determined the particulate phosphorus (PP) divided into organic (POP) and inorganic (PIP) fractions. In this paper, the seasonal variations of the C/Chl.*a*, C/N and PIP/PP ratios of particulate matter were discussed. In summer, Chl.*a* was high, and Chl.*a* and PIP/PP ratios indicated phytoplankton activities were also high. Our dataset also indicated that surface particulate matter contained a large amount of non-phytoplankton particle in the coastal water, even when phytoplankton biomass was high in summer. We found that the PIP/PP ratio was a good indicator to predict the phytoplankton activity and the origin of particulate matter of surface seawater as well as the C/Chl.*a* and C/N ratio.

Keywords : *Seto Inland Sea, Phytoplankton, C/Chl.a ratio, particulate inorganic phosphorus (PIP).*

1. Introduction

A high primary productivity, phytoplankton activity, and phytoplankton growth in coastal waters have been discussed (CLOERN *et al.*, 1995; CLOERN, 1999). C/chlorophyll *a* (Chl.*a*) ratio is an important index to predict primary productivity of phytoplankton (CLOERN *et al.*, 1995; WANG *et al.*, 2013). C/Chl.*a* ratio was reported for cultured phytoplankton (FALKOWSKI *et al.*, 1985; GEIDER, 1987; CLOERN *et al.*, 1995) and field observations (Van LEEUWE and De BAAR, 2000; Le BOUTEILLER *et al.*, 2003; MARANON, 2005; SATHYENDRANATH *et al.*, 2009). Although there are many data of C/Chl.*a* ratios, little is known about the spatial and temporal variations in C/Chl.*a* (WANG *et al.*, 2013). Most of the C/Chl.*a* data in the field observations were mainly studied in open ocean but not many in coastal waters. In addition, there are temporal variations in primary productivity, phytoplankton activities and components of phytoplankton depending on ambient environmental conditions (e.g., CLOERN, 1999; GEIDER and La ROCHE, 2002; ARRIGO, 2005). Here, we investigated seasonal variations of the C/Chl.*a* ratio in Harima-Nada, the Seto Inland Sea, and discuss the phytoplankton activity in the coastal water.

We also measured particulate phosphorus (PP) dividing into organic (POP) and inorganic (PIP) fractions. PP is mainly composed

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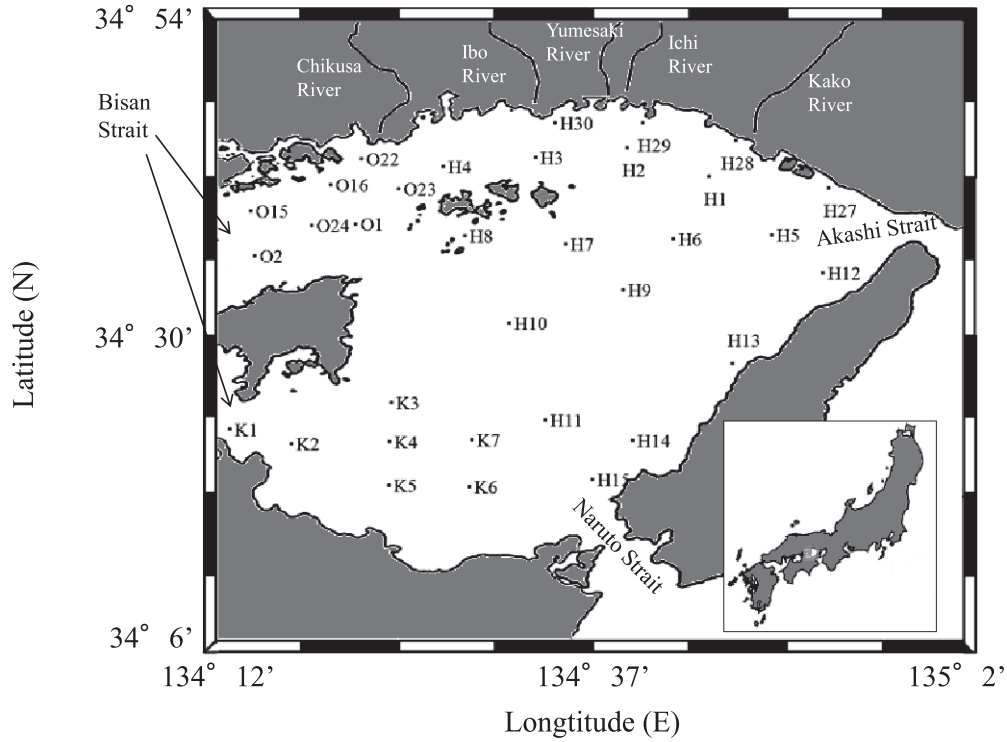

Fig. 1. Sampling stations in Harima-Nada, the Seto Inland Sea, Japan.

of phytoplankton and fresh marine organic matter in the open ocean (YOSHIMURA *et al.*, 2007). However, high concentrations of PIP are often observed in coastal and estuarine waters due to terrigenous input and resuspension of sediment particles, and the distribution of PP shows a slight difference compared with that of Chl.*a* in coastal areas and estuaries (SUZUMURA *et al.*, 2004; LOASSACHAN *et al.*, 2008). Although it has been reported that phosphorus was preferentially remineralized relative to carbon and nitrogen in marine ecosystems (LOH and BAUER, 2000), BENITEZ-NELSON *et al.* (2004) suggested from measurement of PP divided into POP and PIP that POP is not preferentially remineralized relative to particulate organic carbon (POC) and nitrogen (PON). Information obtained in this way of dividing PP into POP and PIP is important for identifying particulate phosphorus behavior. Thus, in the present paper, we suggested that PP and PIP are also useful indexes to predict phytoplankton activities and components of particulate matters in coastal waters. We investigated Chl.*a* , particulate organic carbon (POC), particulate inorganic nitrogen (PON), PP and PIP in the coastal water to reveal seasonal variations in phytoplankton activities and components of particulate matters.

2. Material and Method

2.1 Study site

Harima-Nada is located in the eastern part of the Seto Inland Sea. Its average water depth is about 30 m. This is a semi-enclosed sea connecting to Bisan-Seto in the west, to Osaka Bay in the east, and to Kii Channel in the south, being connected to the Pacific Ocean. The northern part of Harima-Nada has a strong freshwater discharge from several large rivers (Fig. 1). In Harima-Nada, the primary production rate is higher in the Seto Inland Sea next to the highly eutrophicated Osaka Bay and northern Hiroshima Bay (HASHIMOTO and TAKEOKA, 1998;

TADA *et al.*, 1998).

2.2 Sampling

Water samples were collected at 33 stations from all over Harima-Nada (Fig. 1). Observations were conducted 4 times (May, August and November 2011 and February 2012) to see the seasonal variations. In Stn. H27, we could not collect surface water due to Nori (*Pyropia*) cultivation in November and February.

At every sampling station, salinity and water temperature were measured from surface to bottom by a CTD (AAQ1183, JFE Advantech), and surface water was collected with a plastic bucket to measure concentrations of Chl.*a* , PP, PIP, POC and PON. Filter samples were stored in a cooler with ice, and kept frozen (−30°C) until analysis after bringing to the laboratory.

2.3 Chl.*a* determination

Concentrations of Chl.*a* were analyzed by the fluorescence method (HOLM-HANSEN *et al.*, 1965) described in PARSONS *et al.* (1984). Aliquots of water samples were filtered through a Whatman GF/F filter. Filter samples were soaked in 90% acetone in dark at 5°C for 24 h. After soaking, fluorescence of extractions was determined by AU10 (Turner Design) to measure concentrations of Chl.*a*.

2.4 PP and PIP determination

Concentrations of PP and PIP in surface water were analyzed by combustion and colorimetric method (ASPILA *et al.*, 1976; LOH and BAUER, 2000; SUZUMURA *et al.*, 2004). Aliquots of water samples were filtered through a precombusted (500°C for 4 h) Whatman GF/F filter. Filter samples were freeze-dried and then combusted at 550°C for 2 h. Combusted filter samples were then soaked in 1 N HCl at room temperature (ca. 20°C) for 12 h. After soaking, extractions were neutralized by 1 N NaOH and their adsorption was measured through spectrophotometry by V530 (JASCO), using the method of MURPHY and RILEY (1962). The concentrations of PP were calculated by the absorbance of the extractions. Concentrations of PIP were estimated by a method similar to PP measurement but without the combustion process.

2.5 POC and PON determination

Concentrations of POC and PON in surface water were analyzed by dry combustion method. Aliquots of water samples were filtered through a precombusted (500°C for 4 h) Whatman GF/F filter; then, filters were rinsed with 1 N HCl to remove inorganic carbon (HEDGES and STERN, 1984) and were rinsed with distilled water. After freeze-drying, filters were measured for POC and PON using a CHN elemental analyzer (JM10, J-SCIENCE LAB).

2.6 $\Delta\sigma$t

$\Delta\sigma$t was calculated by the difference between σt of the surface and bottom, and used as the indicator of the stratificational strength.

3. Results and discussion

3.1 Seasonal variations of the concentrations of Chl.*a*, POC and phytoplankton C/Chl.*a* ratio

Mean water temperature and $\Delta\sigma$t in each observation was showed in Fig. 2. In the Seto Inland Sea, water mass structure, namely, stratification and vertical mixture of sea water are important for nutrient dynamics, distribution of particulate matter and primary production (HASHIMOTO *et al.*, 1997; KOBAYASHI *et al.*, 2007). In Harima-Nada, surface water temperature ranges approximately from 8°C in February and to 26−28°C between August and early September. In addition, it is well-known that thermal stratification develops from April to August and the water column is vertically mixed from September to March (NISHIKAWA *et al.*, 2007). In our study, the mean water temperature in August were 26.4°C, which marked the highest value in observations. Water temperature also showed the lowest value in February (7.8°C). The values of $\Delta\sigma$t in May and August were also higher and were quite lower in November and February. Judging from water temperatures and the values of $\Delta\sigma$t, water column was mixed in November and February and was stratified in May and August. Thus, we regarded the data in May, August, November and February as representing months in spring, summer, autumn and winter,

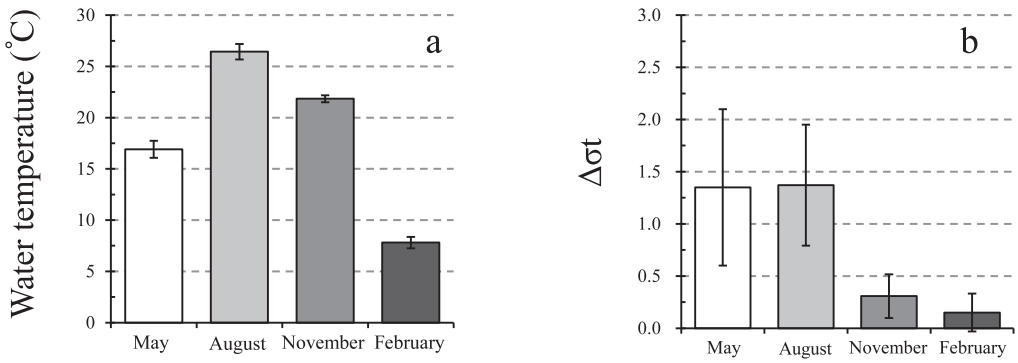

Fig. 2. Seasonal variations in water temperature (a) and $\Delta\sigma t$ (b). Vertical bars represent SD.

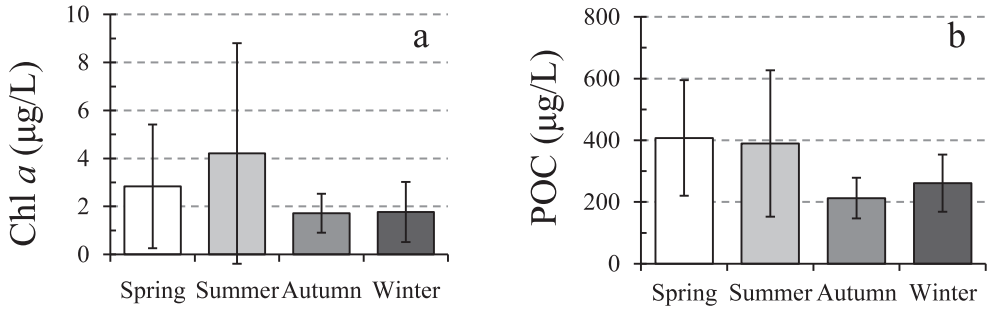

Fig. 3. Seasonal variations in Chl.*a* (a) and POC (b).

respectively.

Chl.*a* varied from 0.377 to 11.6 μg/L in spring, 0.720 to 21.5 μg/L in summer, 0.529 to 3.82 μg/L in autumn, and 0.390 to 5.50 μg/L in winter, respectively. The concentration of POC also varied from 170 to 1120 μg/L in spring, 184 to 1470 μg/L in summer, 125 to 385 μg/L in autumn, and 163 to 549 μg/L in winter, respectively. In the horizontal distributions, high Chl.*a* and POC concentrations were found in the northern coastal area in Harima-Nada in all seasons (the details of this phenomenon will be shown in ASAHI *et al.*, 2014). The seasonal variations of POC showed a similar trend with that of Chl.*a* (Fig. 3). In spring and summer, higher mean Chl.*a* (2.84±2.54, 4.21±4.59 μg/L, respectively) and POC (407±187, 390±237 μg/L, respectively) concentrations were observed, and higher phytoplankton activity can be predicted in spring and summer.

In all seasons, there were positive correlations between Chl.*a* and POC in Harima-Nada (Fig. 4). However, POC may not represent only phytoplankton carbon but detritus, bacteria and other POC. Since it is difficult to divide particulate components into individual phytoplankton carbon and detritus carbon, we cannot directly estimate the concentrations of phytoplankton carbon. Consequently, phytoplankton POC/Chl.*a* ratios (g/g) were estimated from the slope of the regression line of Chl.*a* and POC (TADA *et al.*, 2006).

C/Chl.*a* had a mean value of 60.3 in 4 seasons. The C/Chl.*a* of 44.1 in summer, when Chl.*a* was higher, was clearly lower than those in the other seasons (Figs. 3 and 5). Measurements with phytoplankton grown in culture showed that C/Chl.*a* ranged from 10 to 330

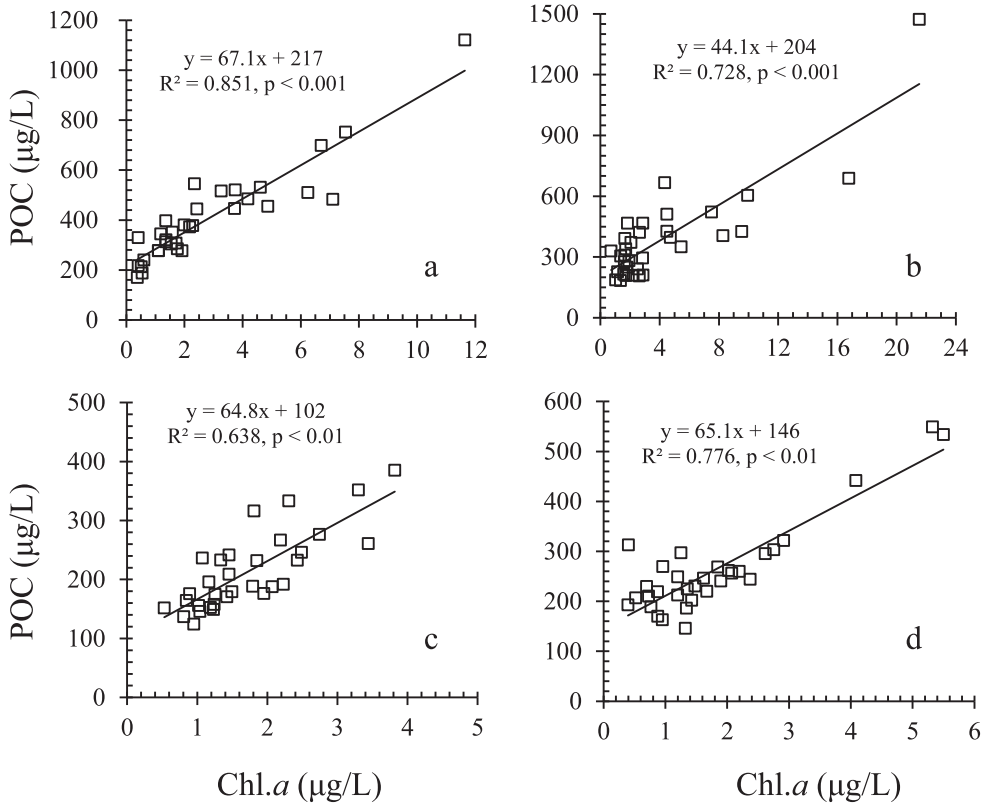

Fig. 4. Scatter plot of POC versus Chl.*a* in spring (a), summer (b), autumn (c) and winter (d).

(Falkowski *et al.*, 1985; Geider, 1987; Cloern *et al.*, 1995). In field observations, the large variation in C/Chl.*a* was also reported to range from 15 to 200 (Van Leeuwe and De Baar, 2000; Le Bouteiller *et al.*, 2003; Maranon, 2005; Sathyendranath *et al.*, 2009). Although it is well-known that such C/Chl.*a* variations are due to the variations of concentrations and molar ratios of nutrients (Strickland, 1960; Antia *et al.*, 1963; Cloern *et al.*, 1995), light conditions (Cloern *et al.*, 1995) and size of a phytoplankton cell (Le Bouteiller *et al.*, 2003), little is known about the spatial and temporal variations in C/Chl.*a* (Wang *et al.*, 2013). Low C/Chl.*a* ratio in summer (44.1) in our study suggested that in Harima-Nada, phytoplankton had a high primary production rate and grew rapidly in summer (Strickland, 1960; Antia *et al.*, 1963). Although C/Chl.*a* ratios in the other seasons in Harima-Nada (64.8 to 67.1) were higher than that in summer (44.1) (Fig. 5), they were still lower than that in the open ocean reported as 105 in the Pacific

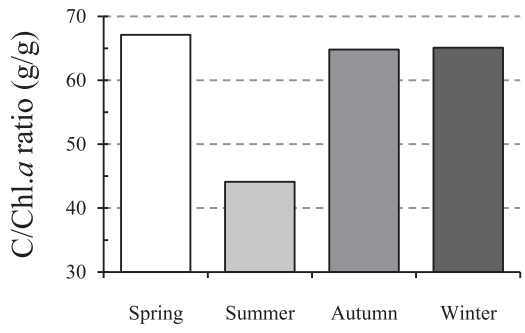

Fig. 5. Seasonal variations in C/Chl.*a* ratio.

and 74 in the Atlantic (WANG *et al.*, 2013). This result indicated that, in costal waters such as Harima-Nada, phytoplankton activity is high throughout the year and particularly higher in summer.

3.2 Particulate C/N ratio

The C/N ratio in Harima-Nada clearly showed a seasonal variation such as the higher values in spring (11±1.4) and summer (8.3±0.90), and the lower values in autumn (7.1±0.97) and winter (6.8±0.37) (Fig. 6). It is well-known that the C/N ratio is one of the good indicators of sources of organic matter (PRAHL *et al.*, 1980; THORNTON and MCMANUS, 1994). The C/N ratio of particles mainly derived from marine organic matter was reported as 6.2－8.8 mol/mol (TAN *et al.*, 1991), being close to the phytoplankton C/N ratio (6.6) represented by the Redfield ratio (REDFIELD *et al.*, 1963).

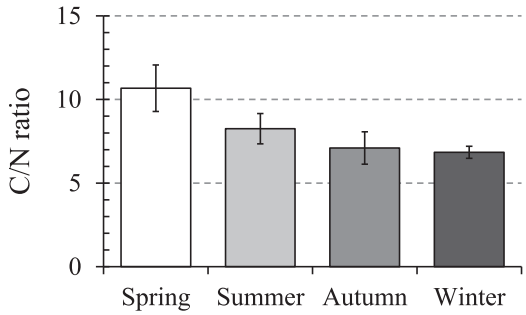

Fig. 6. Seasonal variations in C/N ratios in suspended particulate matter. Vertical bars represent SD.

Meanwhile, the C/N ratio of terrestrial or decomposed organic matter is more than 10 (TAN *et al.*, 1991; THORNTON and MCMANUS, 1994; MEKSUMPUN *et al.*, 2005).

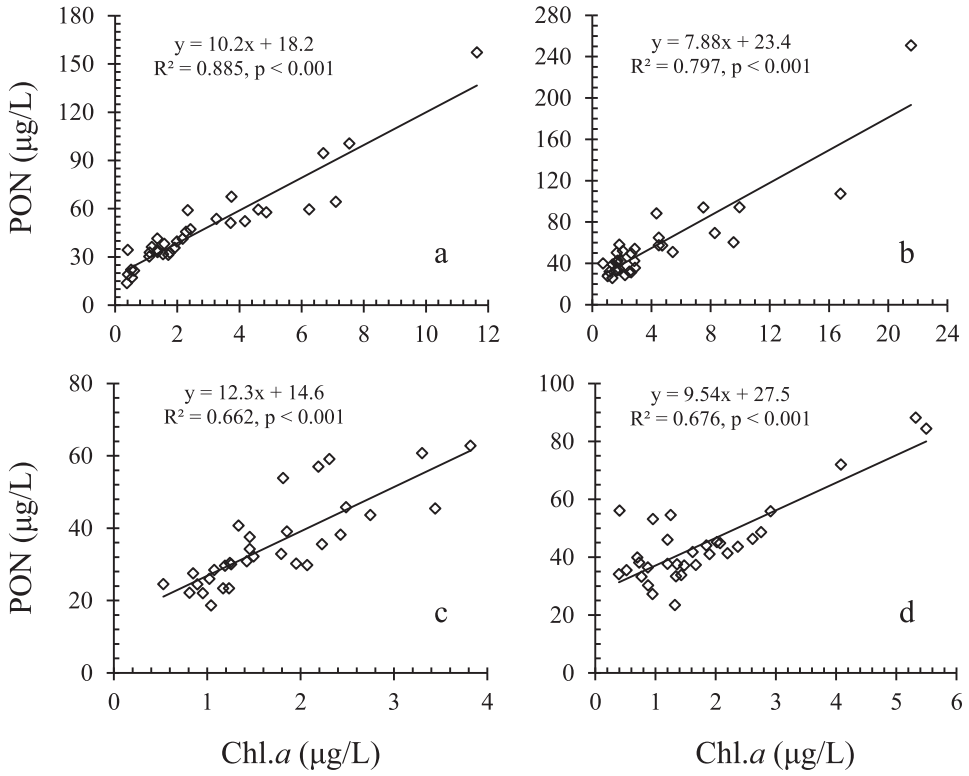

Fig. 7. Scatter plot of PON versus Chl.*a* in spring (a), summer (b), autumn (c) and winter (d).

In our study, it was inconsistent that, in summer, Chl.*a* was high but the C/N ratio was not similar to that of phytoplankton. Also, in autumn and winter, Chl.*a* was lower (Fig. 3a) but the C/N ratios were similar to that of phytoplankton. Then we estimated phytoplankton carbon and nitrogen from the slope of the correlation between Chl.*a* and POC (Fig. 4), and between Chl.*a* and PON (Fig. 7), respectively and regarded that the intercept of the regression line was detritus carbon and nitrogen. The phytoplankton C/N ratio calculated from the slope of these equations (Figs. 4 and 7) ranged from 6.1 to 8.0 and was close to phytoplankton C/N (6.6) (Fig. 8). On the other hand, detritus C/N ratios ranged from 6.2 to 14 (Fig. 8). Hence, phytoplankton C/N ratios were approximately 6.6 through the year but detritus C/N ratios varied beyond the Redfield ratio. Moreover, the detritus matter contributed greatly to the C/N ratio of suspended particles in the surface coastal water even when phytoplankton biomass was higher in summer. This means there was a problem in determining the origin of suspended matter in coastal waters from the C/N ratio alone.

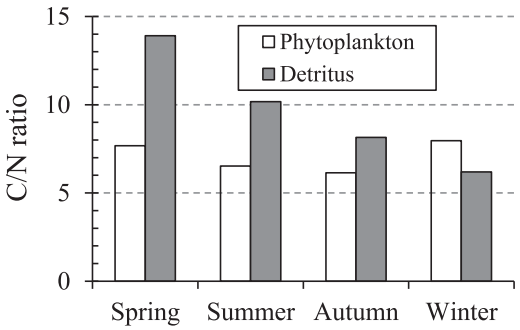

Fig. 8. Seasonal variations in C/N ratios in phytoplankton and detritus. Carbon and nitrogen of the phytoplankton and detritus were calculated from the slope and intercept of the equations between Chl.*a* and POC (Fig. 4), and Chl.*a* and PON (Fig. 7).

3.3 Particulate phosphorus

PP and PIP varied from 1.44 to 19.5 and 0.621 to 5.33 μg/L in spring, 10.5 to 54.9 and 2.17 to 9.92 μg/L in summer, 5.68 to 15.5 and 1.15 and 5.63 μg/L in autumn, and 5.29 to 23.4 and 1.80 to 7.61 μg/L in winter, respectively. In the horizontal distributions, high PP and PIP were found in the northern coastal area in Harima-Nada in all seasons and also high PIP were found in the channel area. More detailed results will be shown in our other paper. We also estimated phytoplankton PIP (g/g) from the slope of the regression line between Chl.*a* and PIP in the same manner as the C/Chl.*a* ratio. In addition, we regarded the intercept of the regression line between Chl.*a* and PIP to be detritus PIP (μg/L). From Fig. 9, we can see phytoplankton PIP/Chl.*a* ratio ranged from 0.284 to 0.791 g/g and detritus PIP 0.800 to 3.18 μg/L.

PIP is composed mostly of a particulate bound form of phosphate which adsorbs minerals and heavy metals such as Fe, Mg or Ca (Lebo, 1991; Ruttenberg, 1992). Our method for analyzing PIP, however, measures the PO_4-P adsorbed on the phytoplankton cell surface, which is acid labile fraction (Sanudo-Wilhelmy *et al.*, 2004; Fu *et al.*, 2005). Therefore, our PIP values were included surface-adsorbed phosphorus on phytoplankton. In the north Pacific, where there is small terrigenous discharge, PIP showed 10－20% PP, and evidenced a significant positive correlation with Chl.*a* (Yoshimura *et al.*, 2007). Since those researchers measured PIP in the same way as in this study, 0.1－0.2 of the PIP/PP ratio could be considered similar with the phytoplankton PIP/PP ratio, and the slope of the regression correlation between concentrations of Chl.*a* and PIP (14.49 nmol PIP/ μg Chl.*a* or 0.449 g PIP/g Chl.*a*, Yoshimura *et al.*, 2007) could be considered the phytoplankton PIP/Chl.*a* ratio.

In Harima-Nada, the phytoplankton PIP (PIP/Chl.*a* ratio, g/g) in spring and summer was lower than that in the open ocean (0.449) and in autumn and winter (Fig. 9). Sanudo-Wilhelmy *et al.* (2004) suggested that actively growing phytoplankton cells hold acid labile phosphorus on their surface and it accounted for up to 30% of total phosphorus, although cells in senescent hold 90% of total phosphorus on their surface. Thus, it is thought that our low PIP/Chl.*a* ratios in spring and summer indicated high phytoplankton activities since the concentrations of Chl.*a* in spring and summer

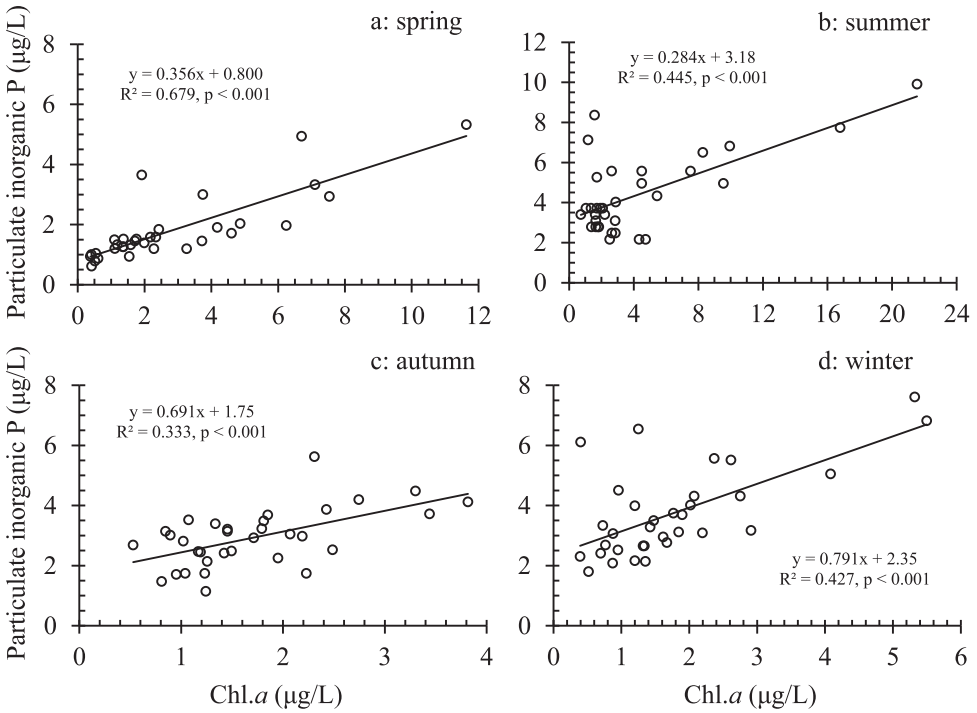

Fig. 9. Scatter plot of PIP versus Chl.*a* in spring (a), summer (b), autumn (c) and winter (d). Each plot area includes the equation of linear regression.

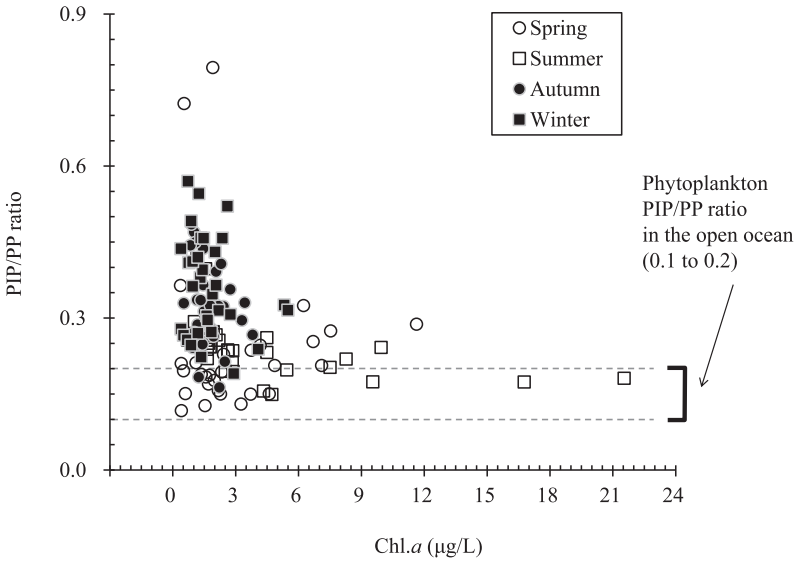

Fig. 10. Scatter plot of PIP/PP ratio versus Chl.*a*. Phytoplankton PIP/PP ratio in open ocean is referenced by Yoshimura *et al.*, 2007.

were high and the C/Chl.*a* ratio in summer was low (Fig. 3).

Although the intercept of the correlation between Chl.*a* and PIP in open ocean was little (1.76 nmol/L=0.0546 μg/L, YOSHIMURA *et al.,* 2007), those in Harima-Nada were high (Fig. 9). This result indicated that there was a considerable detritus contribution to particulate matter in coastal waters; for example, high PIP/PP particles such as terrestrial matter discharged from the river (SUZUMURA *et al.*, 2004; LOASSACHAN *et al.*, 2008).

PIP/PP ratios in Harima-Nada showed a large variation with the changing Chl.*a* and PIP/PP ratios of particulate mater were decreasing and close to the phytoplankton PIP/PP ratio (0.1 to 0.2) with increasing Chl.*a* (Fig. 10). This result also indicated that particulate matters in coastal water contained a high PIP originating from non-phytoplankton particles, and usually showed a higher PIP/PP than in the open ocean. However, when there is high phytoplankton activity and high Chl.*a* concentration, the PIP/PP ratio in coastal water is close to that of phytoplankton.

4. Conclusions

C/Chl.*a* ratio in Harima-Nada indicated higher phytoplankton activity in surface coastal water, particularly in summer. The PIP/Chl.*a* ratio also indicated higher phytoplankton activity in spring and summer when the surface coastal water showed high Chl.*a*. However, there the amounts of PIP derived from non-phytoplankton particles such as detritus in the surface coastal water was so large that the concentration of PIP in coastal water was higher than that in the open ocean.

Particulate components in surface coastal water differed from both phytoplankton and those of the open ocean. Detritus, with a high C/N ratio and high PIP/PP ratio, may contribute to surface particles in coastal water in such a large amount that surface particulate matter show a high C/N ratio in spring and summer, and high PIP/PP ratio in autumn and winter. C/N ratios in our study were high in spring and summer, but low in autumn and winter, suggesting that it is difficult to predict the component of coastal particulate matter from only the C/N ratio because of the large amount of detritus in coastal water as mentioned above. C/N ratios are sometimes different from other source indicators such as stable isotopic ratio (e.g., THORNTON and MCMANUS, 1994; MEKSUMPUN *et al.*, 2005; LAMB *et al.*, 2006) since particulate matter including decomposed phytoplankton has a high C/N ratio but low isotope carbon (MEKSUMPUN *et al.*, 2005). Thus, we consider that PIP and PIP/PP ratio are good indicators for identifying particle components and phytoplankton activity as well as C/Chl.*a* ratio and C/N ratio.

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