

Characterization of Selenium in the Deep Ocean Water Pumped up at Muroto, Japan

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Abstract

Muroto deep ocean water has been pumped up from a point within a 3 km distance from the coast and at depths of 320 m or 344 m in the sea, in Muroto, Kochi, Japan. In this study, in order to clarify the characteristics of selenium in the Muroto deep ocean water, total selenium and selenium speciation were investigated. Both inorganic and organic selenium were detected, and two distinct features of the selenium were discovered. Compared to ocean surface and river waters, (1) Muroto deep ocean water contained a high level of total selenium, and (2) less toxic selenium species, namely selenite and organic selenide were dominant. The total selenium content of Muroto deep ocean water was in the same range of the North Pacific Ocean. The majority of selenite and selenate occurred in the dissolved fraction; however, around 60 % of the organic selenide was found in the particulate fraction (particles diameter > 0.22 μm). Selenium contained in the organic and inorganic particulates accounted for 55 and 45 % of the total particulates selenium, respectively. Moreover, free and combined seleno-amino acids accounted for more than 70 % of the dissolved organic selenide.

Key Words: *Deep ocean water, selenium, selenite, selenate, seleno-amino acids.*

1. Introduction

Deep ocean water, which is defined as the seawater below a depth of ca. 200 m, possesses the typical features of rich nutrients, low temperature and cleanliness. Attention has been given to deep ocean water for its potential as a new resource for the generation of electricity, aquaculture, cold water agriculture, air conditioning, industrial cooling, and desalination in recent years (Craven and Sullivan, 1998). Muroto deep ocean water has been pumped up from a point within a 3 km distance from the coast and at depth of 320 m and 344 m in the

sea. There often occurs upwelling events of water mass onto the continental shelf at a depths of 500 ~ 1000 m. About 900 t of deep ocean water has been pumped up every day, and has been used for drinking water production, food processing, and skin lotion production, etc. However, the actual possible effect of the deep ocean water in the above applications has not been demonstrated yet. One of the reasons can be attributed to the lack of knowledge of the basic characteristics of the deep ocean water including trace elements involved. Therefore, we focused on the amount and speciation of selenium in the deep ocean water in this study.

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Selenium is the key element of glutathione-peroxidase, an enzyme preventing peroxidation of membranes in organisms. It is reported that selenium can improve the immunity of the organisms and also has an anti-cancer effect. Insufficient uptake of selenium can cause Keshan disease, a kind of heart disease in humans; and muscular dystrophy in live stock (Kang, 1989). On the other hand, selenium is a toxic element; an excess uptake of selenium can cause disorder of nail or hair for humans (Yang *et al.*, 1983).

Dissolved selenium in seawater has three oxidation states, selenate, selenite and selenide. Sugimura *et al.* (1976) showed both selenite and selenate to be present in oxic seawater, with higher concentrations in deep waters than at the surface. Measures and Burton (1980) and Measures *et al.* (1980) presented the first oceanographically consistent vertical profiles of selenate and selenite in the Atlantic and Pacific Oceans. They concluded that selenium displays nutrient-type behavior. Cutter (1982) found that selenite and selenate concentrations decrease in the suboxic zone and are at the detection limits in anoxic water in Saanich Inlet. The major selenium species in the reducing waters was operationally identified as dissolved organic selenide, and most of them were found to be associated with dissolved amino acids (Cutter, 1982). Thus, dissolved selenide may exist in the form of seleno-amino acids in certain environments. In addition to regeneration, the particulate uptake of selenium has also been studied. Wrench and Measures (1982) presented data indicating the preferential uptake of selenite over selenate during seasonal plankton blooms in a Nova Scotian fjord.

Nakaguchi *et al.* (2001) indicated that selenite reveals nutrient-type behavior; while selenate

concentration has no relation to the biological activity in the surface water, but it is largely dependent on water mass-movement (in the Eastern Indian Sea). It was also pointed out, that organic selenide was distributed not only in the surface water, but also substantially present in deep water.

The objectives of this study are to elucidate selenium concentration, chemical species and seasonal variation in Muroto deep ocean water, and determine selenium species in fluxing particulate material to clarify the basic characteristics of Muroto deep ocean water.

2. Study Sites and Methods

Samples and sampling sites are shown in Fig. 1 and Table 1. Surface and deep ocean waters were obtained from the sluice gates of Kochi Prefectural Deep Seawater Laboratory, Muroto, Kochi, which pumped up the waters from a depth of 0.5 m and 344 m, respectively. The ocean waters were collected once a month from February to December, 2001. River waters were taken at a depth of 0.5 m from Niyodo, Kagami and Monobe rivers in Kochi prefecture on 8th and 9th of Jun, 2001. All water samples were collected in 2 – 5 liter plastic bottles. The pH (Horiba F-21) and electric conductivity (EC) (TOA, CM-14P) were measured immediately after bringing the samples to the laboratory (Table 2). The samples for the determination of selenate, selenite and organic selenide analysis were passed through 0.45 μm membrane filters, and then stored in plastic bottles in a refrigerator (4°C). Analyses were completed within 1 week after sampling. The deep ocean waters that were collected from 5th to 16th every day in August, 2002 were passed through 0.45 μm and 0.22 μm membrane filters. Unfiltered samples,

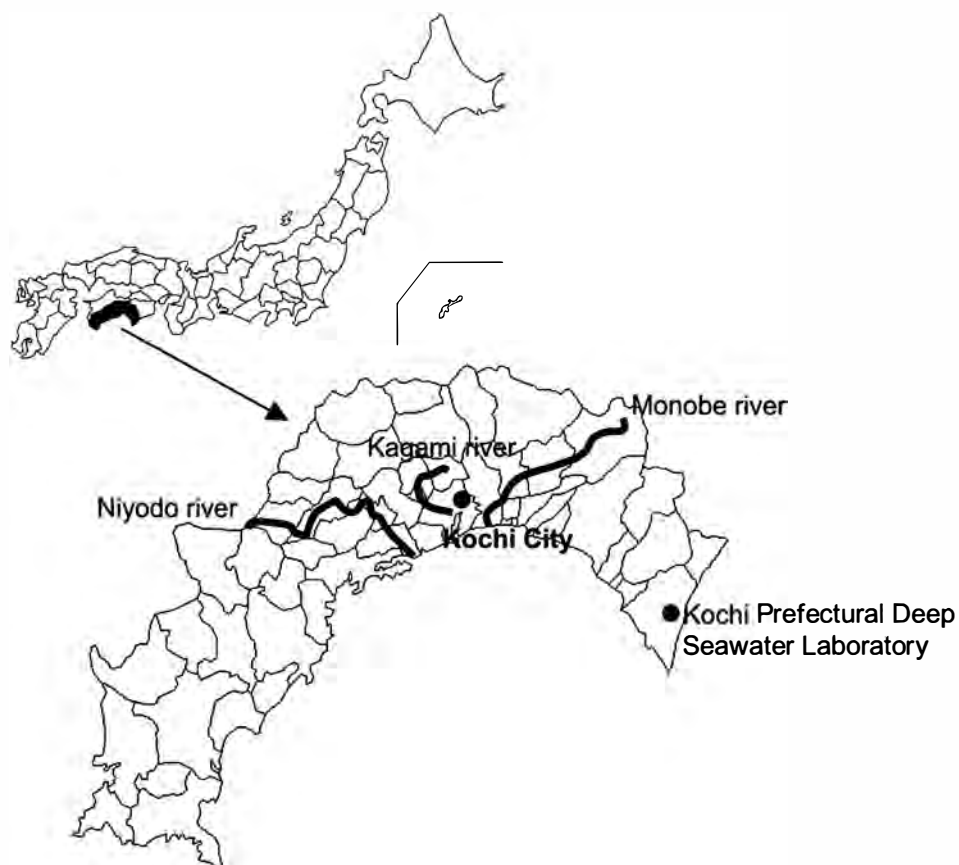


Fig. 1 Location of water samples of deep ocean water, surface seawater and river waters in Kochi Prefecture, Japan

Table 1 Water samples and sampling sites

<i>Ocean Waters</i>	<i>Sampling Sites</i>	<i>Depth (m)</i>
Surface	Muroto, Kochi	0.5
Deep	Muroto, Kochi	320, 344
<i>River Waters</i>	<i>Sampling Sites</i>	<i>Distance from Coast (km)</i>
Niyodo River	Ino, Kochi	10
Kagami River	Asakura, Kochi	10
Monobe River	Monobe, Kochi	3

Table 2 pH and electric conductivity of the ocean waters determined in 2001

Items \ Samples	Ocean Waters		River Waters		
	Deep	Surface	Niyodo	Kagami	Monobe
pH	7.65±0.092	8.10±0.055	7.13	7.66	8.82
EC (mSm ⁻¹)	6630±80	6480±111	8.73	8.50	13.7

0.45 μm filtrates and 0.22 μm filtrates were subjected to the analysis of total selenium contents and its speciation.

The determination methods for selenate, selenite and organic selenide have been reported previously (Yamada *et al.*, 1990). The technique includes fluorescence formation, high performance liquid chromatography and fluorescence detection. Selenite was determined after reacting with 2, 3-diaminonaphthalene. Selenate + selenite were determined by boiling the samples in 0.1 % potassium bromide and 1.5 M hydrochloric acid for 20 min at 100 °C, followed by the procedure for selenite determination. The first step to determine the total dissolved selenium was potassium persulfate decomposition, and then followed by the procedure of selenate + selenite determination. The difference between the total dissolved selenium and selenate + selenite was allocated as dissolved organic selenide.

Selenium in particle was evaluated using the procedures reported by Yamada *et al.* (1987). The deep ocean waters, including unfiltered waters, and 0.45 μm and 0.22 μm filtrates were concentrated 5 times on a water bath at 50 °C. One ml of the concentrated samples was decomposed with a mixture of 0.5 ml hydrofluoric, nitric and perchloric acids in Teflon vessels. To the resultant solution, 2 ml of 6 M hydrochloric acid was added, and the whole solution was heated in a water bath at 100 °C for 15 min. Then, the solution was subjected to the same procedure as selenate + selenite determination. Selenium in total particles was determined from the differences in the total selenium contents between the unfiltered samples and < 0.22 μm filtrates decomposed by hydrofluoric, nitric and perchloric acids. Selenium in organic particles was determined by potassium persulfate decomposition,

i.e. differences between the unfiltered samples and the < 0.22 μm filtrates. Finally, selenium in inorganic particles was calculated by subtracting selenium in the organic particles from selenium in the total particles.

Furthermore, in order to characterize the dissolved organic selenide, both free and combined seleno-amino acids dissolved in seawater and river water were measured using the method proposed by Siegel and Degens (1966). A 40 ml of filtrates passed through the 0.22 μm filter were hydrolyzed in 6 M HCl for 24 hours. Then, the solution was heated on a hot plate at 60 °C to remove the HCl. After dissolving the contents in deionized water and adjusting the pH to 9 with 0.1 M NaOH, the solution was passed through a column filled with copper sulfate treated Chelex 100 resin. This column chelates free amines, which can then be eluted with 1.5M ammonium hydroxide solution. The total selenium content of the solution was measured.

Selenium in all samples was measured by HPLC with fluorescence detection (Yamada *et al.*, 1987). Detection limit and repeatability of this method were 3 pg and 1.9 - 3.9 %, respectively.

3. Results and Discussion

3.1 Dissolved selenium speciation and concentration in the ocean and river waters

Fig.2 shows the dissolved selenium species and their concentration in the ocean and river waters. Total selenium content of Muroto deep ocean water averages $2.3 \pm 0.19 \text{ nmol L}^{-1}$, and was significantly higher than that in the surface ocean water ($1.6 \pm 0.31 \text{ nmol L}^{-1}$). It was about 2 times higher than that in the river waters ($1.0 - 1.3 \text{ nmol L}^{-1}$). Selenate, selenite and the organic selenide were detected both in the ocean

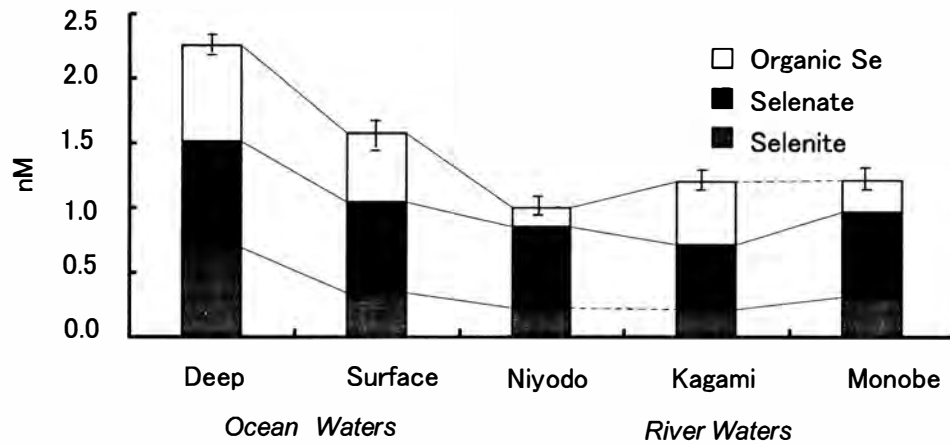


Fig. 2 Dissolved Se species and concentrations of the ocean and river waters

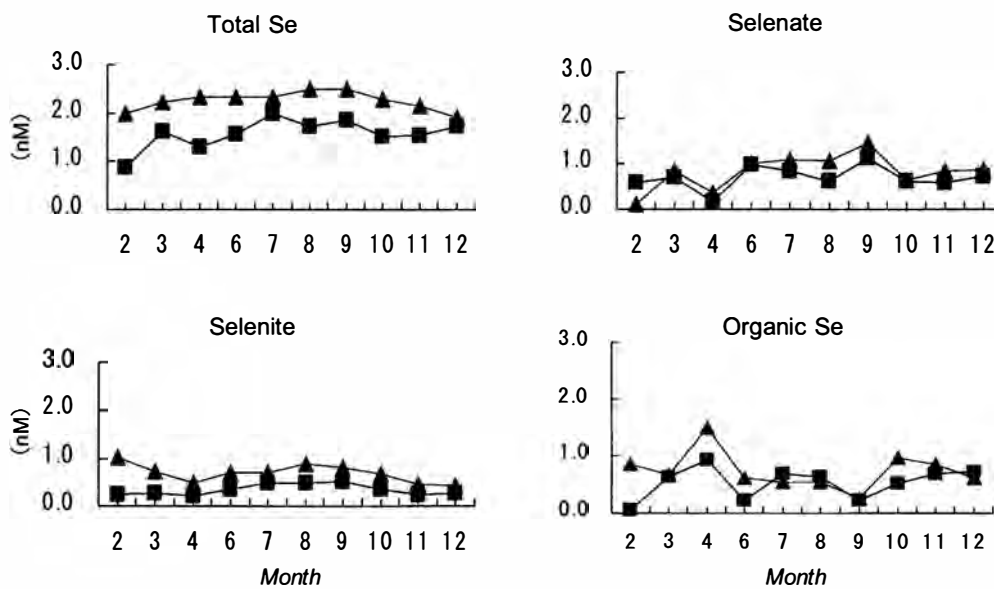


Fig. 3 Monthly changes of Se species and concentration in deep ocean water and surface seawater at Muroto in 2001 (▲ deep ocean water; ■ surface seawater)

and river waters. Selenate is the most abundant species, followed by selenite or organic selenide. In the surface ocean water or river waters, the concentration ratio of selenate to selenite ranged from 2.1 – 2.7, but it was as low as 1.2 (0.83 – 1.6) in the deep ocean water. However, the selenite concentration in the deep ocean water is more than twice that of the surface ocean water or river waters.

From these results, it is concluded that the deep ocean water has two obvious features on species and distribution of selenium compared to ocean surface and river waters; (1) it contains

a high level of total selenium, and (2) it contains a higher level of the less toxic selenium species, i.e., selenite and organic selenide, although selenate concentration is high as that of surface seawater.

3.2 Fluctuation of selenium species and concentrations in Muroto ocean waters

Fig. 3 shows the fluctuation of selenium species and concentrations in Muroto surface and deep ocean waters in 2001. Over the course of one year examination, no distinct seasonal changes were observed neither in the surface

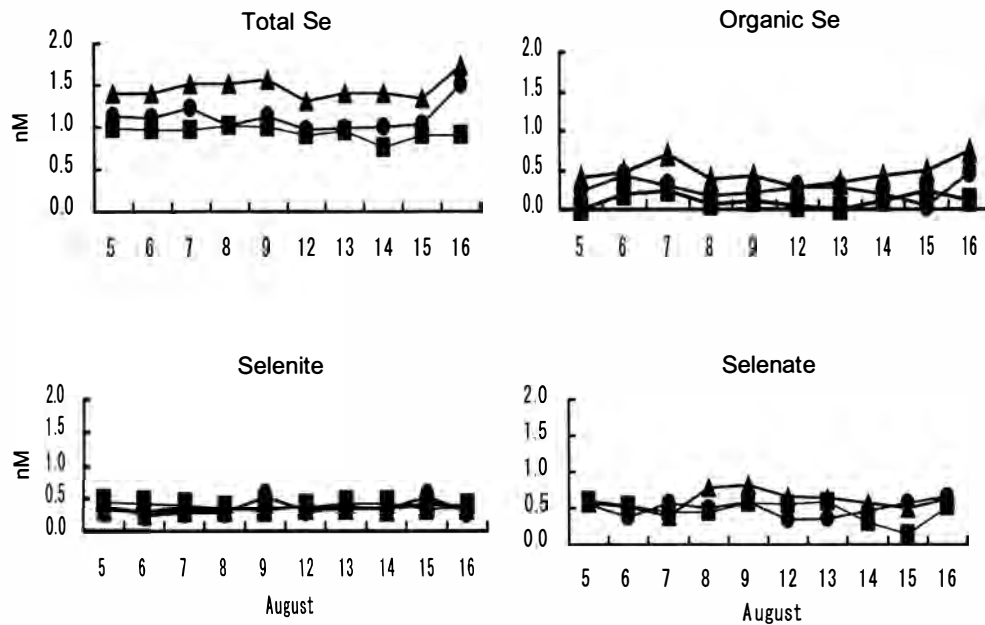


Fig. 4 Se concentrations in the Muroto deep ocean water filtered with different pore size filters in 2002 (▲ unfiltered; ● 0.45 μm ; ■ 0.22 μm)

nor deep waters.

Total selenium concentration of Muroto surface and deep ocean waters collected in 2001 averaged 1.6 nmol L^{-1} ($0.89 - 2.0 \text{ nmol L}^{-1}$) and 2.3 nmol L^{-1} ($1.9 - 2.5 \text{ nmol L}^{-1}$), respectively. It appears that total selenium concentrations in open ocean waters are well below the 3 nmol L^{-1} level (Siu & Berman, 1987). Measures *et al.* (1983) concluded that the Atlantic, Pacific, and Indian Oceans all exhibit similar vertical distributions of selenium which resembled those of phosphate and silicate, namely, concentration increased with an increase in depth. Data taken from the middle layer (500 to 1000 m) of North Pacific Ocean showed that total selenium concentrations were in the range of $1.5 - 2.0 \text{ nmol L}^{-1}$ (Sugimura *et al.*, 1976). Thus, the total selenium concentration of Muroto deep ocean water collected in 2001 was in the same range as the value reported for the North Pacific Ocean. It is also in the same range as the East tropical Pacific Ocean waters (Cutter and Bruland, 1984). However, the samples collected in August, 2002

contained a lower level of total selenium as shown in Fig. 4. Reason for these results is unclear. One possibility is that Muroto deep ocean water might show a long term fluctuation.

Total concentrations of selenium and selenite were higher in the deep ocean water than those in the surface water throughout the year, while no statistical differences (t test) were found in the concentrations of selenate or organic selenide (Fig. 3). While the results of the total selenium, selenite and selenate are similar to those in other ocean basin (Measures *et al.*, 1983; Cutter and Bruland, 1984; Cutter and Cutter, 1995; Nakaguchi *et al.*, 2001), distribution of organic selenide is considerably different. Cutter (1992) reported that organic selenide is only found in surface waters. Data of the Eastern Indian Sea, Bay of Bengal, Andaman Sea, South China Sea and Sulu Sea showed that organic selenide concentration increased in deep waters (Nakaguchi *et al.*, 2001).

Sugimura *et al.* (1976) showed both selenite and selenate to be present in oxic seawater, with

higher concentrations in deep waters than at the surface. Wrench and Measures (1982) indicated the preferential uptake of selenite over selenate during seasonal plankton blooms in a Nova Scotia fjord. They pointed out that microorganisms may select the lowest available oxidation state because less chemical energy is then required to maintain the reductive process linking selenium to organic molecules. Interestingly, selenate is the form of selenium most readily taken up by terrestrial plants (Gissel-Nielsen and Bisbjerg, 1979). Moreover, Wrench and Measures (1982) also found that the decay of the plankton bloom was marked by a rapid regeneration of the dissolved nutrients including selenite. In Muroto ocean waters, it is presumed that plankton preferentially absorbed selenite in the surface water; therefore selenite was found to have a lower concentration in the surface water. Another cause for the elevated selenite concentration in deep water might be the decomposition of planktons precipitated from the surface.

3.3 Dissolved and particulate selenium in Muroto deep ocean water

Muroto deep ocean water samples collected in August, 2002 were filtered with 0.45 and 0.22 μm membrane filters. The concentrations of total selenium, selenate, selenite and organic selenide in the filtrates and unfiltered water samples were measured, respectively. The results are shown in Fig. 4. The total selenium concentration decreased in the order of unfiltered water < 0.45 μm filtrate < 0.22 μm filtrate. The results of speciation showed that no significant differences were found in selenate and selenite concentrations (t test), but the organic selenide content increased in the order of unfiltered water > 0.45 μm filtrate > 0.22 μm

filtrate. A 64 % (52 – 70 %) of the total selenium occurred as dissolved and 36 % (30 – 48 %) as particulate. More than 90 % (58 – 100 %) of selenite and around 80 % (70 – 100 %) of selenate exist as dissolved forms. This is because the pH of the deep ocean water is 7.65; therefore, the adsorption of selenate or selenite to particles is extremely weak. Generally, if the pH is higher than 6.2, most of the selenate and selenite adsorbed on inorganic particles desorbs quickly (Merrill *et al.*, 1986).

In unfiltered water, as much as 77 % of the organic selenide occurred as particulate form. Even in the 0.45 μm filtrate, about 60 % of the organic selenide existed in particulate form (particle size 0.22 – 0.45 μm). However, a part of the total selenium evaluated as being the dissolved organic selenide (operationally defined in 0.45 μm filtrate and unfiltered waters) might be the particulate selenium. Since the samples were decomposed with potassium persulfate in this experiment, it is considered that the particulate selenium measured here was in the form of organic particle. Cutter and Bruland (1984) reported that organic selenide makes up about 80 % of the total dissolved selenium in surface waters in the eastern tropical North Pacific Ocean. They filtered the water samples through 0.30 μm membrane filters. The so-called dissolved organic selenium or selenide possibly includes selenium in fine organic particles. The fine organic particles were considered as microorganism and fragment of organisms.

Results of selenium concentration in Muroto deep ocean water decomposed with HF-HClO₄-HNO₃ or potassium persulfate are shown in Table 3. The total dissolved selenium (< 0.22 μm) was found to be nearly the same in the two decomposition methods, $0.89 \pm 0.030 \text{ nmol L}^{-1}$ in the former and $0.95 \pm 0.080 \text{ nmol L}^{-1}$ in the

Table 3 Se contents of the particles of Muroto deep ocean water in 2002

Particle Sizes \ Treatments	HF-HClO ₄ -HNO ₃ decomposition	K ₂ S ₂ O ₈ decomposition	Se in inorganic particles
< 0.22 μm (nM) (A)	0.89 ± 0.030	0.95 ± 0.080	—
Non-filtration (nM) (B)	1.6 ± 0.16	1.4 ± 0.070	—
> 0.22 μm (nM) (B)–(A)	0.73* (C)	0.40** (D)	0.33 (C)–(D)

* Se content of total particles. ** Se content of organic particles; (n = 3)

Table 4 Contents and ratios of seleno-amino acids in ocean and river waters

Contents of ratios	Deep ocean	Surface ocean	River
Organic Se (nM) (A)	0.45 ± 0.064	0.32 ± 0.065	0.22 ± 0.014
Seleno-amino acids (nM) (B)	0.34 ± 0.027	0.22 ± 0.012	0.096 ± 0.016
Ratio (B/A) (%)	75	70	44

latter. However, the total selenium in the unfiltered water samples revealed considerably different levels, $1.6 \pm 0.16 \text{ nmol L}^{-1}$ with the HF-HClO₄-HNO₃ decomposition method, and $1.4 \pm 0.070 \text{ nmol L}^{-1}$ with the potassium persulfate decomposition method. Total particulate selenium was 0.73 nmol L^{-1} , accounted for 46 % of the total selenium in infiltration water. The organic and inorganic particulate selenium levels were 0.40 and 0.33 nmol L^{-1} , and these accounted 55 and 45 % of the total particulate selenium, respectively. Thus, the Muroto deep ocean water has two origins of the particulate selenium, organic and inorganic particles.

3.4 Characterization of the dissolved organic selenide in the ocean and river waters

Organic selenide in seawater is considered to occur as volatile dimethyl selenide and trimethyl selenide, and free or combined seleno-amino acids contained in peptides and protein (Nakaguchi *et al.*, 2001). As revealed in Table 4, seleno-amino acids (both free and combined)

accounted for around 70 % of the total dissolved organic selenide in the deep and surface ocean waters, but it was only 44 % in the river water. Cutter (1982) reported that at least an average of 65 % of the dissolved organic selenide could be accounted for as selenium in the total amino acids fraction (samples collected from 175 to 190 m depth). Organic selenide was the predominant species in surface waters in the North and South Pacific Oceans (Cutter and Bruland, 1984). It was noted that organic selenide is almost exclusively found in the youngest deep water masses in the North Atlantic Ocean (Cutter and Cutter, 1998). Data taken from the Eastern Indian Sea reported by Nakaguchi *et al.* (2001) showed that the dissolved organic selenide was found both in the surface and deep waters (0 to 6000 m). Results of this study are similar to the data of Cutter (1982) in the chemical form and to those of Nakaguchi *et al.* (2001) in vertical distribution. The possible reason of organic selenide in Muroto deep ocean water might be because it contains young water masses.

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室戸海洋深層水のセレン特性

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要 旨

海洋深層水は新しい機能性資源として注目を集めている。今では、水産物の養殖、食品製造、化粧品開発、医療・健康や農業など様々な分野で利用されるようになり、多くの成果が報告されている。しかし、その機能メカニズムは必ずしも十分に解明されていない。その原因の一つは微量元素を含む海洋深層水の基礎的性質の解明が欠如していることである。それゆえ、本研究では生体中で過酸化を防止し、免疫能力を高める微量元素セレンを取り上げ、室戸海洋深層水のセレン特性について検討を行なった。

室戸海洋深層水の全セレン含量 (2.3 ± 0.19 nM) は表層水 (1.6 ± 0.31 nM) や河川水 (1.0~1.3 nM) に比べて顕著に高いことが判った。溶存形態別にみると、表層水や河川水でセレン酸イオンの割合 (>50%) が高いのに対して、深層水ではセレン酸イオン、亜セレン酸イオンと有機態セレンの割合がほぼ同レベルであった。表層水や河川水と比較して、深層水の亜セレン酸イオンと有機態セレンの割合が高かった。海洋表層では亜セレン酸イオンが生物に選択的に吸収されるとの報告があり、そのために深層水で亜セレン酸イオン濃度が相対的に高くなっているのではないかと考えられる。また、有機態セレンは分解されずに深層に沈降してくる生物遺体などに由来するものと推察される。1年間の変動を調べたところ、深層水の全セレン濃度の変動(変動係数, 8.6%)は表層水(19.7%)より小さく、比較的安定であった。

深層水のセレンを粒径別にみると、溶存態セレンが最も多く(64%)、続いて粒径 $>0.45 \mu\text{m}$ (24%)と $0.22 \sim 0.45 \mu\text{m}$ の懸濁態(12%)の順となり、懸濁態の割合がかなり高いことが分かった。また、粒径が大きくなるにつれて有機態画分のセレン含量が高くなるのに対し、セレン酸と亜セレン酸イオン含量はほとんど変化が認められなかった。これは深層水のpHが7.8と高いために、無機態セレンが粒子に吸着されることなく、溶存態で存在するためと考えられた。懸濁態の内訳は生物破片に含まれるセレンが55%、無機粒子に含まれるセレンが45%を占め、いずれも細かい粒子に多く含まれていた。また、溶存有機態セレンの約70%が遊離アミノ酸あるいはペプチドに含まれることが明らかになった。

以上の結果より、室戸海洋深層水のセレン特性は表層水や河川水と大きく異なることが明らかとなった。しかし、他の海域の深層水と比較すると、セレンの濃度と形態は同様な傾向を示した。

キーワード：海洋深層水、セレン、亜セレン酸イオン、セレン酸イオン、セレノアミノ酸