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1 **Seasonal Changes of Arsenic Speciation in Lake Waters in Relation to**
2 **Eutrophication**

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1 Abstract:

2 In this study, the influence of eutrophication on arsenic speciation in lake waters was
3 investigated. Surface water samples ($n = 1-10$) were collected from 18 lakes in Japan during July
4 2007 and February 2008. The lakes were classified into mesotrophic (7 lakes) and eutrophic (11
5 lakes) based on the total phosphate (T-P) and chlorophyll-a (Chl-a) concentrations in water column.
6 Inorganic, methylated and ultraviolet-labile fractions of arsenic species were determined by
7 combining hydride generation atomic absorption spectrometry with ultraviolet irradiation.
8 Organoarsenicals (mainly methylated and ultraviolet-labile fractions) comprised 30-60% of the total
9 arsenic in most lakes during summer. On the other hand, inorganic arsenic species (As(III+V))
10 dominates (about 60-85%) during winter. The occurrence of ultraviolet-labile fractions of arsenic was
11 higher in eutrophic lakes than those in mesotrophic lakes in both seasons. The concentration of
12 dimethyl arsenic (DMAA) was high in eutrophic lakes during winter; and in mesotrophic lakes
13 during summer. The results suggest that the conversion of As(III+V) to more complicated
14 organoarsenicals occurred frequently in eutrophic lakes compared to that in mesotrophic lakes, which
15 is thought to be the influence of biological activity in the water column. The distribution of arsenic
16 species were well correlated with phosphate concentrations than those of Chl-a. This might be due to
17 the competitive uptake of As(V) and phosphate by phytoplankton. The organoarsenicals
18 (OrgAs)/As(V) ratio was higher at low phosphate concentration indicating that conversion of As(V)
19 to OrgAs species was more active in phosphate-exhausted lakes with high phytoplankton density.

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22 **Keywords:** Arsenic, Eutrophication, Organoarsenicals, Ultraviolet photolytic decomposition, Lake
23 water, Chlorophyll-a

1 **1. Introduction:**

2 Arsenic (As) is widely distributed in aquatic environments in various forms (Cullen and
3 Reimer, 1989). Arsenate ($\text{AsO}(\text{OH})_3$; As(V)) is the thermodynamically stable state in oxic waters,
4 while arsenite ($\text{As}(\text{OH})_3$; As(III)) is predominant in reduced redox conditions (Andreae, 1986; Cullen
5 and Reimer, 1989). In natural waters, the dominant inorganoarsenicals (iAs) are incorporated into
6 microorganisms such as phytoplankton, and are converted to methylarsenicals and/or more high order
7 organoarsenicals such as arsenosugar (Francesconi et al., 1996; Maher, 1984). In addition, the
8 organoarsenicals are mineralized to iAs and methylarsenicals by bacteria (Hanaoka et al., 1995).

9 In natural waters, the cycling of As species would depend on the bioactivity of organisms
10 (Cullen and Reimer, 1989; Sanders, 1980). Microorganisms produce methylarsenicals in natural
11 waters (Sanders and Riedel, 1993), which exhibit seasonal cycle with maximum concentrations of
12 methylarsenicals in summer (Hasegawa et al., 1999; Howard et al., 1995; Sohrin et al., 1997a).
13 Methylarsenicals are considered to be produced by phytoplankton and organisms of higher trophic
14 levels as a detoxification mechanism (Edmonds and Francesconi, 1987). Sanders and Riedel (1993)
15 reported the correlation between As(III)/methylarsenicals and chlorophyll-a concentrations and/or
16 phytoplankton density, while Howard et al. (1995) found that the seasonal changes of DMAA
17 concentration is related to the temperature rather than the biological activity of phytoplankton. The
18 bulk of other organoarsenicals are also found in organisms (Maeda, 1994). The arsenosugars are
19 usually found in algae and arsenobetaine is the predominant form in marine animals (Edmonds and
20 Francesconi, 1987; Francesconi and Kuehnelt, 2002). The degradation and mineralization of
21 organoarsenic compounds are thought to depend mostly on bacterial activities, which influence the
22 As cycling in aquatic environment (Kaise et al., 1987; Maki et al., 2005).

1 Arsenic metabolism in aquatic organisms results in the occurrence of thermodynamically
2 unstable arsenite and methylarsenic compounds in natural waters. The inorganic forms (As(V) and
3 As(III)) and the methylated forms (methylarsonic acid ($\text{CH}_3\text{AsO}(\text{OH})_2$); MMAA(V) and
4 dimethylarsinic acid ($((\text{CH}_3)_2\text{AsO}(\text{OH}))$); DMAA(V)) are the main species of As in natural waters
5 (Cullen and Reimer, 1989). The bulk of the total dissolved As is iAs in seawater (Peterson and
6 Carpenter, 1983) and in freshwater (Kuhn and Sigg, 1993; Seyler and Martin, 1989). Although the
7 predominant form of methylarsenicals is consistently DMAA(V) followed by MMAA(V), the
8 existence of trivalent methylarsenic species in the environment has also been reported (Hasegawa,
9 1996; Hasegawa et al., 1994; Sohrin et al., 1997a).

10 In general, eutrophication is a phenomenon involving an increased concentration of nutrient
11 salts, which promotes the multiplication of primary producers such as phytoplankton, in inland water.
12 Lakes progress through the oligotrophic, mesotrophic and eutrophic process in the natural
13 environment, and these transitions are very slow. Recently, the transition speed became faster
14 because of discharged pollutants and nutrients from human activities, and the eutrophication affects
15 the As circulation in lakes (Sohrin et al., 1997a).

16 In this study, As speciation in surface water was determined by hydride generation atomic
17 absorption spectrometry equipped with cold trap (CT-HG-AAS). The CT-HG-AAS is widely used in
18 the analysis of As compound, such as As(V), As(III), MMAA(V) and DMAA(V) (Braman et al.,
19 1977; Hasegawa et al., 1994). In this technique, As is reduced to hydride forms such as arsine, and is
20 then transported to the atomization part at boiling point. There are others infinitesimal As species
21 compared to inorgano- and methyl-arsenicals in natural waters, however, they are not hydride
22 reactive forms. Therefore, it is necessary for the organoarsenicals to be converted to hydride reactive
23 forms using UV irradiation (Hasegawa et al., 1999). Although the distribution and speciation of As in

1 eutrophic and mesotrophic lakes were discussed in our previous article (Hasegawa et al., 2009), it
2 was difficult to interpret the influence of eutrophication on As speciation because of limited data
3 from a less number of lakes. In the present study, the correlation between As speciation and
4 eutrophication are inferred based on samples collected from 18 lakes in Japan during July 2007 and
5 February 2008. Although the lakes were classified into oligotrophic, mesotrophic and eutrophic, the
6 oligotrophic and the mesotrophic lakes were combined together, and discussed collectively for
7 enhancing understanding.

8

9 **2. Experimental**

10 **2.1. Sample Collection and Pretreatment**

11 We collected surface water samples ($n = 1-10$) from 18 lakes during July, 2007 to February,
12 2008 (Fig. 1). Chlorophyll-a (Chl-a), water temperature and pH were measured with a multiple water
13 quality analyzer (model 6600, YSI) on site. Various basic water parameters and sampling information
14 of the lakes are presented in Table 1.

15 The samples were collected within 0.2 m of the water surface near the shore of the lakes, and
16 were well saturated with oxygen. For analysis of As and nutrients, the samples were filtered with
17 0.45 μm (HA type, Millipore) filters immediately after collection. Both filtered and unfiltered
18 samples were acidified to pH 2.0 by the addition of 1.0 M hydrochloric acid (HCl), and stored in
19 refrigerator until analysis.

20

21 **2.2. Reagents**

22 Stock solutions (10^{-2} M) for the determination of As compounds were prepared by dissolving
23 the corresponding sodium salts ((CH)₃AsO₃Na₂ was prepared by Quick's method (Hasegawa et al.,

1 1994), and NaAsO₂, Na₂HAsO₄ and (CH₃)₂AsO₂Na were obtained from Nacalai Tesque, Japan) in
2 0.1 M sodium hydroxide. They were diluted to the desired concentrations just before use. Sodium
3 borohydride (Kanto Chemical, Japan) was used for hydride generation. A 3% (w/v) sodium
4 borohydride solution, stabilized in 10⁻² M sodium hydroxide solution, was prepared daily. Other
5 reagents were of analytical grade or better.

6

7 2.3. Arsenic analysis

8 Analysis of inorganic and methylarsenicals was performed by a modified technique of
9 hydride generation method (CT-HG-AAS) using an apparatus and materials identical to those
10 described in our previous paper (Hasegawa et al., 1994). The detection limits were 0.11 nM and 0.14
11 nM for As(V+III) and MMAA, respectively (3 times the standard deviation of the blank), and the
12 precision of five replicate determinations were 2.1% for inorganic As and 5.1% for DMAA (a relative
13 standard deviation) at 1.0 nM with a 30 ml sample size.

14 Organoarsenic species, except for methylarsenicals, can be classified into different fractions
15 according to their lability to the photochemical degradation; UV-As and UV-DMAA (Hasegawa et
16 al., 1999). UV-As and UV-DMAA contain arsenosugar, arsenolipid, arsenocoline, arsenobetaine etc.
17 Samples (30 ml) were acidified to pH 2.0 using 1.0 M HCl, and introduced into 50-ml quartz test
18 tubes that were capped with natural rubber septums. They were then irradiated with a 400 W high-
19 pressure mercury lamp (Sigemi, AHH-400S). Aliquots were taken at selected time intervals. Arsenic
20 analysis of the digests was performed with CT-HG-AAS as described above.

21 The UV-DMAA was stepwise decomposed to As(V) through DMAA(V) by ultraviolet
22 irradiation with a time. The UV-As was transformed to As(V) directly. The detail of the
23 organoarsenic speciation is shown elsewhere (Hasegawa et al., 2009). The UV-As and UV-DMAA

1 were estimated from the concentration changes of As(V+III) and DMAA during the ultraviolet
2 irradiation by a non-linear least-squares computation, respectively (Hasegawa et al., 1999).

3

4 **3. Results and Discussion**

5 **3.1. Trophic classification of lakes**

6 Eutrophication is the natural ageing process of lakes. This is a very slow process, which
7 ultimately transforms aquatic environments into terrestrial habitats, begins with the addition of
8 nutrients into the system. These nutrients, in turn, stimulate the growth of microscopic free-floating
9 aquatic plants known as phytoplankton. The term 'eutrophication' is also used to describe the human
10 induced process where by human activity such as agriculture, use of fertilizers and changes in land
11 surrounding aquatic environments, accelerates the growth of phytoplankton.

12 Nitrogen and phosphorus usually control the growth of phytoplankton in the aquatic
13 environment. Based on the amount of phytoplankton growth and the concentration of nutrients, the
14 degree of eutrophication in aquatic environments can be classified as oligotrophic, mesotrophic,
15 eutrophic, or hypereutrophic. In the present study, water samples from a total of 18 lakes in Japan
16 were collected for the investigation (Fig. 1). The lakes were classified into oligotrophic, mesotrophic,
17 and eutrophic lakes to assess the influence of eutrophication on As cycling and speciation (Fig. 2).
18 The Chl-a and phosphate are two important parameters of trophic state of aquatic systems (Carpenter
19 et al., 1996; Smith et al., 1999). Although there are several trophic state indices (TSI) for the
20 assessment of lake water quality (Carlson, 1977; Carlson, 1980; Smith et al., 1999; Stefanou et al.,
21 2000), the trophic classification of the lakes was performed on the basis of chlorophyll-a and total
22 phosphate (T-P) concentrations (Figs. 3A, 3B) according to the OECD's criteria (Karydis and
23 Coccossis, 1990; Moriki and Karydis, 1994; OECD, 1982) as; oligotrophic lakes (mean Chl-a; < 2.5

1 $\mu\text{g/l}$, mean T-P; $< 0.32 \mu\text{mol/l}$), mesotrophic lakes (mean Chl-a; $2.5\text{-}8 \mu\text{g/l}$, mean T-P; $0.32\text{-}1.13$
2 $\mu\text{mol/l}$), and eutrophic lakes (mean Chl-a; $8 > \mu\text{g/l}$, mean T-P; $>1.13 \mu\text{mol/l}$).

3 Oligotrophic environments are characterized by clear waters, little suspended organic matter
4 or sediment, and low primary production (phytoplankton growth). Mesotrophic environments have
5 higher nutrient inputs and rates of primary production. Eutrophic environments have extremely high
6 nutrient concentrations and biological productivity. Hypereutrophic environments are characterized
7 by murky, highly productive waters in which many clear water species cannot survive (Allen and
8 James, 1972; Genkai-Kato and Carpenter, 2005; Moncreiff, 2002; Smith et al., 2006; Smith et al.,
9 1999). However, the results of the oligotrophic and the mesotrophic lakes did not differed
10 significantly from each other in the present study. Thus, among the 18, 7 were classified as
11 mesotrophic lakes and 11 as eutrophic lakes.

12 Figure 3 shows mean Chl-a and T-P concentrations in mesotrophic and eutrophic lakes. The
13 Chl-a content in eutrophic lakes was clearly higher than that in mesotrophic lakes. Among eutrophic
14 lakes the highest Chl-a content was recorded in lake Suigetsu ($81.2 \mu\text{g L}^{-1}$) whereas the lowest was in
15 lake Nishino ($9.71 \mu\text{g L}^{-1}$). The Chl-a content in mesotrophic lakes was below $10 \mu\text{g L}^{-1}$. The trend
16 of T-P concentration in lake waters followed the same pattern of Chl-a. However, the T-P
17 concentration in surface water of eutrophic lakes was significantly higher than that in mesotrophic
18 lakes. The highest T-P was found in lake Kahuku ($4.14 \mu\text{g L}^{-1}$) while the lowest was in lake Suwa
19 ($1.12 \mu\text{g L}^{-1}$). Although the T-P concentration in Lake Hiruga was higher than that in Lake Suwa, the
20 lake Hiruga was considered as mesotrophic and Lake Suwa as eutrophic lake on the basis of Chl-a
21 content.

22

23 3.2. Seasonal distribution of As species in lake waters

1 Figures 4A and 4B show the distribution of As species in the 18 lakes. Both in eutrophic and
2 mesotrophic lakes, the total As concentrations in the summer were higher than in the winter. These
3 trends of As occurrence and distribution in lake waters were also reported previously (Creelius,
4 1975; Hasegawa et al., 2009; Takamatsu and Kawashima, 1985). Inorganic As was released into the
5 water from the sediments in the bottom layer under anaerobic conditions in summer, while As(V) was
6 adsorbed onto Fe/Mn oxides and then settled down into the sediments in winter.

7 Organoarsenicals (mainly methylarsenicals and UV-labile fractions) comprised about 30-60%
8 and 30-70% of the total As in mesotrophic and eutrophic lakes, respectively, in summer (Fig. 5). A
9 large difference was observed between mesotrophic and eutrophic lakes in the composition of
10 organoarsenicals in summer. The ultraviolet-labile fraction comprised about 30-40% of the total
11 organoarsenicals in the mesotrophic lakes, with the exception of two lakes. In contrast, it comprised
12 about 50-90% of the total organoarsenicals in eutrophic lakes. The results indicate that the conversion
13 of inorganic and methylarsenicals to more complex organoarsenicals is higher in the eutrophic lakes
14 in the summer.

15 The high concentrations of organoarsenicals during summer might be due to the conversion of
16 inorganic As into organoarsenic compounds by aquatic organisms. Some aquatic organisms uptake
17 inorganic As and excrete DMAA in freshwater (Cullen and Reimer, 1989; Hasegawa et al., 2001;
18 Maeda, 1994). A substantial amount of UV-labile fractions of As were also detected in the water of
19 most lakes during summer (Fig. 4A). On the other hand, about 85% of the total As was As(V+III),
20 the amount of UV-As was minimal, and the UV-As, UV-DMAA and other organoarsenicals were
21 totally absent or below the instrumental limit of detection during winter season (Fig. 4B).

22 Results of the present study show that without some exceptions, the concentrations of UV-As
23 were higher usually in high-eutrophic lakes (Lake Suga, Lake Suigetsu, Lake Biwa (southern basin),

1 Lake Kita, and Lake Kahuku) than those in low-eutrophic lakes during summer (Fig. 4A). On the
2 other hand, the concentrations of DMAA were higher in low-eutrophic lakes during winter than those
3 in summer (Fig. 4B). Results suggest that the occurrence of UV-degradable fractions of As is higher
4 in high eutrophic lakes and the production of DMAA is related to the water temperature in low
5 eutrophic lakes. The occurrence of DMAA has been reported in other geographical areas (Anderson
6 and Bruland, 1991; Hasegawa et al., 2009), and in laboratory experiments (Hasegawa et al., 2001).
7 Frequent occurrence of DMAA than that of As(III) has been reported by Howard et al. (1995) at
8 higher water temperatures. Sohrin et al. (1997b) also observed correlation between the concentration
9 of DMAA and water temperature in Lake Biwa, Japan. Hasegawa (1996) reported that the
10 concentration of DMAA follows the rise of water temperature in estuarine waters. The microbial
11 activities, which influence the methylarsenicals' production (Maki et al., 2005), are related to the
12 water temperature. The DMAA is produced by bacterial degradation of complex organoarsenicals in
13 the suspended solids, which is subsequently released into the water column. Thus bacterial activity is
14 responsible for the increase of DMAA concentration in surface water (Hasegawa et al., 2009). Higher
15 concentrations of DMAA might also be resulted from the long-term accumulation of DMAA excreted
16 by phytoplankton.

17

18 **3.3. Effect of eutrophication on As distribution in lake waters**

19 In freshwater systems the proportions of As species vary with the scope of anthropogenic
20 input and biological activity. A number of freshwater organisms have been reported to contain
21 organoarsenic compounds. Production of methylarsenicals or bio-transformation of organoarsenicals
22 from As(V) have been reported in literatures ((Hasegawa et al., 2001; Maeda, 1994). The decrease of
23 total As concentration and the relative increase of methylarsenicals with the increasing trophic level

1 have been observed in most of the aquatic food chains (Kuehnelt and Goessler, 2003).

2 Total As concentrations in surface water of eutrophic lakes were higher than those in
3 mesotrophic lakes in both seasons. Figures 4A and 4B show that the proportion of UV-labile fractions
4 of As were higher in eutrophic lakes compared to those in mesotrophic lakes. The increase of UV-
5 labile fractions of As well correlates with the phosphate and Chl-a concentrations (Figs. 3A, 3B).
6 Since phosphate and Chl-a are important determinants of eutrophication, the results suggest that the
7 occurrences of UV-As and UV-DMAA in lake waters are influenced by eutrophication. The sum of
8 UV-labile As and DMAA concentrations in surface water of eutrophic lakes was increased in summer
9 (Fig. 4A). The result is consistent with the previous report of Hasegawa et al. (2009). High
10 concentration of Chl-a in most eutrophic lakes in the present study (Fig. 3A) reveal that the source of
11 UV-labile As and methylarsenicals would be the direct production of phytoplankton, or the
12 decomposition of organic matter containing complex organoarsenic compounds by microorganisms
13 or sunlight. As the photochemical degradation by sunlight does not contribute to the production of
14 DMAA in lake waters (Hasegawa et al., 1999), the possible reason for DMAA production would be
15 the degradation of complex organoarsenic compounds by microbial activity.

16 The concentrations of UV-As and UV-DMAA was correlated with that of DMAA in
17 eutrophic lakes. The UV-As and UV-DMAA appeared in summer though their occurrences decreased
18 in winter (Fig. 4A, 4B). The production of UV-As and UV-DMAA would be related to the biological
19 activity as the similar manner of DMAA (Hasegawa et al., 2009). Although DMAA and As(V+III)
20 could be released from the particles of organic matter by ultraviolet irradiation, the UV-As(III+V)
21 and UV-DMAA fractions would mainly consist of complex organoarsenic compounds which were
22 synthesized in phytoplankton and other freshwater organisms.

1 The ratios of UV-fractions of As were higher in eutrophic lakes during summer, while those
2 of As(III+V) were high in mesotrophic lakes during winter. On the other hand, the ratios of methyl
3 arsenicals (DMAA and MMAA) were almost same in eutrophic lakes during winter and in
4 mesotrophic lakes during summer (Fig. 5). It can be elucidated by the biological/microbial activities
5 in the lake waters. The eutrophication increased the microbial biomass and biosynthesis of complex
6 organoarsenic compounds in the lakes, which results in the degradation of DMAA and other
7 organoarsenic compounds. Moreover, the concentration of methylarsenic species was significantly
8 lower in eutrophic lakes in summer than those in winter, though the amount of As(III+V) remain
9 unchanged (Fig. 5). The result also suggests the degradation of methylarsenicals (mainly DMAA) in
10 eutrophic lakes during summer.

11

12 **3.4. Relationship between As and phosphate or Chl-a in lake waters**

13 Figure 6 shows the correlation of Chl-a and phosphate (PO_4^{3-}) concentrations with the occurrences of
14 As(III) and organoarsenicals (sum of methylarsenicals, UV-As, and UV-DMAA) in the lake waters.
15 The distribution of arsenic species was well correlated with phosphate (PO_4^{3-}) concentrations than
16 those of Chl-a. As(III)/As(V) ratio was low in phosphate-rich lakes ($> 0.2 \mu\text{mol L}^{-1}$) in Fig. 6C. The
17 result indicates that the As(V) intake by phytoplankton and other aquatic organisms was limited in
18 phosphate-rich lakes. Since PO_4 and As(V) have similar chemical properties (Cullen and Reimer,
19 1989; Sohrin et al., 1997a), phytoplankton intakes As(V) as well as phosphate, competitively. In
20 phosphate sufficient condition, organisms usually intake phosphate instead of As(V) for their
21 metabolic activity (Meharg and Hartley-Whitaker, 2002; Rahman et al., 2008; Wang et al., 2002). On
22 the other hand, high As(III)/As(V) and OrgAs/As(V) ratios were observed in phosphate-poor lakes in
23 Fig. 6D. The correlation explains the influence of phosphate on the conversion of As(V) to As(III)

1 and organoarsenic species by phytoplankton. The conversion of As(V) to As(III) and organoarsenicals
2 was more active in phosphate-exhausted lakes. Thus, the exhaustion of phosphate was considered to
3 be essential for the conversion of As(V). These results are consistent with those of [Sohrin et al](#)
4 [\(1997a\)](#) and [Froelich et al \(1985\)](#).

5 Dissolved oxygen (DO) also plays an important role in the occurrence of As(III) in lake water.
6 Biological activities, which use a substantial amount of dissolved oxygen, are higher in eutrophic
7 lakes while the activities are limited in mesotrophic lakes ([Ærtebjerg et al., 2003](#); [Genkai-Kato and](#)
8 [Carpenter, 2005](#); [Kennish and Townsend, 2007](#); [Moncreiff, 2002](#); [OECD, 1982](#); [Smith et al., 2006](#);
9 [Smith et al., 1999](#)). Therefore, the amount of dissolved oxygen in the bottom layers decreased in
10 eutrophic lakes compared to that in mesotrophic lakes ([Meyer-Reil and Köster, 2000](#)). Comparatively
11 high oxic condition in mesotrophic lakes favor the oxidation of As(III) to As(V) resulting high
12 concentration of As(V).

13

14 **4. Conclusions:**

15 Eutrophication promotes the biological activity in lake water, which has significant effect on
16 arsenic cycling in aquatic systems. From the correlation between arsenic speciation and
17 eutrophication state of 18 lakes in Japan, it was observed that the percentage of organoarsenicals was
18 about 30-60% of the total arsenic in most of the lakes in the summer, and there was not much
19 difference in the occurrence of arsenic speciation between oligotrophic and eutrophic lakes. The ratio
20 of methylarsenicals was higher in oligotrophic and mesotrophic lakes, while the ratio of the
21 ultraviolet-labile fraction was higher in eutrophic lakes. Thus, it can be elucidated the conversion of
22 inorganic and methylarsenicals to more complicated organoarsenicals is higher in eutrophic lakes
23 compared to that in mesotrophic or oligotrophic lakes.

1

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6 **References:**

- 7 Ærtebjerg G, Andersen JH, Hansen OS. Nutrients and eutrophication in Danish marine waters: a
8 challenge for science and management. Ministry of the Environment; Danish Environmental
9 Protection Agency & National Environmental Research Institute, 2003.
- 10 Allen HE, James RK. Nutrients in natural waters. John Wiley and Sons, Inc., New York, 1972, pp.
11 457.
- 12 Anderson LCD, Bruland KW. Biogeochemistry of arsenic in natural waters: the importance of
13 methylated species. Environ. Sci. Technol. 1991;25:420-427.
- 14 Andreae MO. Organic compounds in the environment. Longman, New York., 1986.
- 15 Braman RS, Johnson DL, Foreback CC, Ammons JM, Bricker JL. Separation and determination of
16 nanogram amounts of inorganic arsenic and methylarsenic compounds. Anal. Chem.
17 1977;49:621-625.
- 18 Carlson RE. A trophic state index for lakes. Limnol. Oceanogr. 1977;22:361-369.
- 19 Carlson RE. More complications in the chlorophyll-Secchi disk relationship. Limnol. Oceanogr.
20 1980;25:379-382.
- 21 Carpenter SR, Kitchell JF, Cottingham KL, Schindler DE, Christense DL, Post DM, et al.
22 Chlorophyll variability, nutrient input, and grazing: Evidence from whole- lake experiments.
23 Ecology 1996;77:725-735.

- 1 Crecelius EA. The geochemical cycle of arsenic in lake Washington and its relation to other elements.
2 *Limnol. Oceanogr.* 1975;20:441-451.
- 3 Cullen WR, Reimer KJ. Arsenic speciation in the environment. *Chem. Rev.* 1989;89:713-764.
- 4 Edmonds J, Francesconi K. Transformations of arsenic in the marine environment. *Cell. Mol. Life*
5 *Sci.* 1987;43:553-557.
- 6 Francesconi KA, Edmonds JS, Sykes AG. Arsenic and marine organisms. *Adv. Inorg. Chem. Volume*
7 *44.* Academic Press, 1996, pp. 147-189.
- 8 Francesconi KA, Kuehnelt D. Arsenic compounds in the environment. In: Frankenberger WT,
9 Dekker JM, editors. *Environmental Chemistry of Arsenic*, New York, 2002.
- 10 Froelich PN, Kaul LW, Byrd JT, Andreae MO, Roe KK. Arsenic, barium, germanium, tin,
11 dimethylsulfide and nutrient biogeochemistry in Charlotte Harbor, Florida, a phosphorus-
12 enriched estuary. *Estuar. Coast. Shelf Sci.* 1985;20:239-264.
- 13 Genkai-Kato M, Carpenter SR. Eutrophication due to phosphorus recycling in relation to lake
14 morphometry, temperature, and macrophytes. *Ecology* 2005;86:210-219.
- 15 Hanaoka K, Nakamura O, Ohno H, Tagawa S, Kaise T. Degradation of arsenobetaine to inorganic
16 arsenic by bacteria in seawater. *Hydrobiologia* 1995;316:75-80.
- 17 Hasegawa H. Seasonal changes in methylarsenic distribution in Tosa Bay and Uranouchi Inlet. *Appl.*
18 *Organomet. Chem.* 1996;10:733-740.
- 19 Hasegawa H, Matsui M, Okamura S, Hojo M, Iwasaki N, Sohrin Y. Arsenic speciation including
20 'hidden' arsenic in natural waters. *Appl. Organomet. Chem.* 1999;13:113-119.
- 21 Hasegawa H, Rahman MA, Matsuda T, Kitahara T, Maki T, Ueda K. Effect of eutrophication on the
22 distribution of arsenic species in eutrophic and mesotrophic lakes. *Sci. Total Environ.*
23 2009;407:1418-1425.

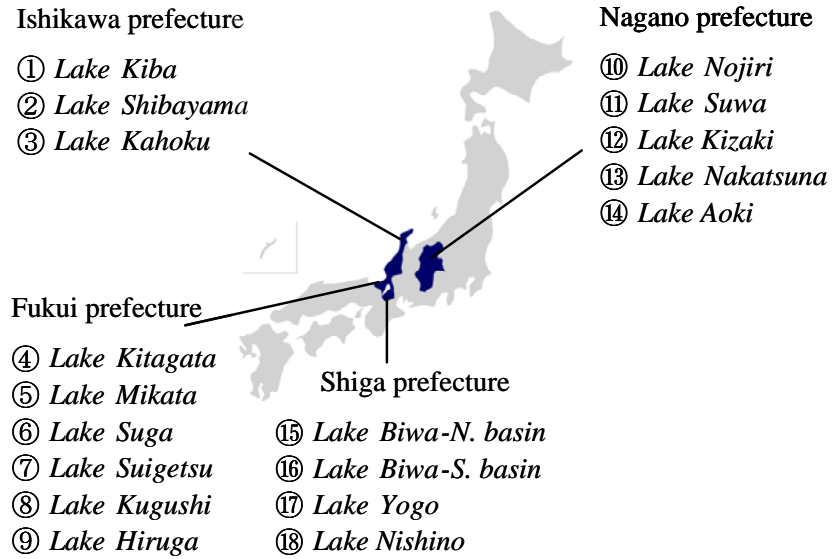
- 1 Hasegawa H, Sohrin Y, Matsui M, Hojo M, Kawashimai M. Speciation of arsenic in natural waters
2 by solvent extraction and hydride generation atomic absorption spectrometry. *Anal. Chem.*
3 1994;66:3247-3252.
- 4 Hasegawa H, Sohrin Y, Seki K, Sato M, Norisuye K, Naito K, et al. Biosynthesis and release of
5 methylarsenic compounds during the growth of freshwater algae. *Chemosphere* 2001;43:265-
6 272.
- 7 Howard AG, Comber SDW, Kifle D, Antai EE, Purdie DA. Arsenic speciation and seasonal changes
8 in nutrient availability and micro-plankton abundance in Southampton water, U.K. *Estuar.*
9 *Coast. Shelf Sci.* 1995;40:435-450.
- 10 Kaise T, Hanaoka K, Tagawa S. The formation of trimethylarsine oxide from arsenobetaine by
11 biodegradation with marine microorganisms. *Chemosphere* 1987;16:2551-2558.
- 12 Karydis M, Coccossis H. Use of multiple criteria for eutrophication assessment of coastal waters.
13 *Environ. Monit. Assess.* 1990;14:89-100.
- 14 Kennish MJ, Townsend AR. Nutrient enrichment and estuarine eutrophication. *Ecol. Appl.*
15 2007;17:S1-S2.
- 16 Kuehnelt D, Goessler W. *Organoarsenic Compounds in the Terrestrial Environment*. New York: John
17 Wiley, 2003.
- 18 Kuhn A, Sigg L. Arsenic cycling in eutrophic lake Greifen, Switzerland: Influence of seasonal redox
19 processes. *Limnol. Oceanogr.* 1993;38:1052-1059.
- 20 Maeda S. *Biotransformation of Arsenic in Freshwater Environment*. New York: John Wiley, 1994.
- 21 Maher W. Arsenic in marine organisms. *Biol. Trace Elem. Res.* 1984;6:159-164.
- 22 Maki T, Hasegawa H, Ueda K. Seasonal dynamics of dimethylarsinic-acid-decomposing bacteria
23 dominating in Lake Kahokugata. *Appl. Organomet. Chem.* 2005;19:231-238.

- 1 Meharg AA, Hartley-Whitaker J. Arsenic uptake and metabolism in arsenic resistant and nonresistant
2 plant species. *New Phytol.* 2002;154:29-43.
- 3 Meyer-Reil L-A, Köster M. Eutrophication of marine waters: Effects on benthic microbial
4 communities. *Mar. Pollut. Bull.* 2000;41:255-263.
- 5 Moncreiff CA. Eutrophication processes: Northwest Florida estuary responses. *Ecology* 2002;83:588-
6 589.
- 7 Moriki A, Karydis M. Application of multicriteria choice-methods in assessing eutrophication.
8 *Environ. Monit. Assess.* 1994;33:1-18.
- 9 OECD. Eutrophication of waters. Monitoring, assessment and control. Tech. Rep. OECD
10 (Organization for Economic Cooperation and Development). Organization for Economic Co-
11 operation and Development (OECD), Paris, 1982, pp. 156.
- 12 Peterson ML, Carpenter R. Biogeochemical processes affecting total arsenic and arsenic species
13 distributions in an intermittently anoxic fjord. *Mar. Chem.* 1983;12:295-321.
- 14 Rahman MA, Hasegawa H, Ueda K, Maki T, Rahman MM. Arsenic uptake by aquatic macrophyte
15 *Spirodela polyrhiza* L.: Interactions with phosphate and iron. *J. Hazard. Mater.* 2008;160:356-
16 361.
- 17 Sanders JG. Arsenic cycling in marine systems. *Mar. Environ. Res.* 1980;3:257-266.
- 18 Sanders JG, Riedel GF. Trace element transformation during the development of an estuarine algal
19 bloom. *Estuaries* 1993;16:521-532.
- 20 Seyler P, Martin JM. Biogeochemical processes affecting arsenic species distribution in a
21 permanently stratified lake. *Environ. Sci. Technol.* 1989;23:1258-1263.
- 22 Smith VH, Joye SB, Howarth RW. Eutrophication of freshwater and marine ecosystems. *Limnol.*
23 *Oceanogr.* 2006;51:351-355.

- 1 Smith VH, Tilman GD, Nekola JC. Eutrophication: impacts of excess nutrient inputs on freshwater,
2 marine, and terrestrial ecosystems. *Environ. Pollut.* 1999;100:179-196.
- 3 Sohrin Y, Matsui M, Kawashima M, Hojo M, Hasegawa H. Arsenic biogeochemistry affected by
4 eutrophication in Lake Biwa, Japan. *Environ. Sci. Technol.* 1997a;31:2712-2720.
- 5 Sohrin Y, Matsui M, Kawashima M, Hojo M, Hasegawa H. Arsenic Biogeochemistry Affected by
6 Eutrophication in Lake Biwa, Japan. In: Kanehisa M, Sato N, Yoko T, editors. *ICR Annual*
7 *Report 1997*. 4. Institute for Chemical Research, Kyoto University, Kyoto, Japan, 1997b, pp.
8 14-15.
- 9 Stefanou P, Tsirtsis G, Karydis M. Nutrient scaling for assessing eutrophication: The development of
10 a simulated normal distribution. *Ecol. Appl.* 2000;10:303-309.
- 11 Takamatsu TM, Kawashima MK. The role of Mn^{2+} -rich hydrous manganese oxide in the
12 accumulation of arsenic in lake sediments. *Water Res.* 1985;19:1029-1032.
- 13 Wang J, Zhao FJ, Meharg AA, Raab A, Feldmann J, McGrath SP. Mechanisms of arsenic
14 hyperaccumulation in *Pteris vittata*: Uptake kinetics, interactions with phosphate, and arsenic
15 speciation. *Plant Physiol.* 2002;130:1552-1561.
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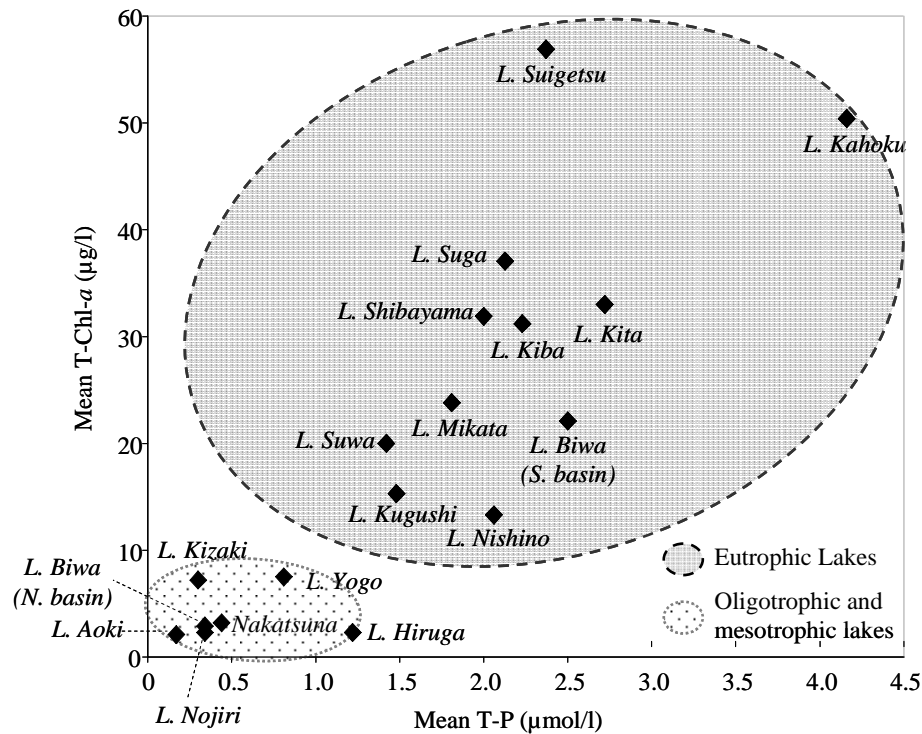
1 Table 1: The table shows various basic water parameters and the sampling information of the lakes studied. In the table, summer and
 2 winter are of the year 2007 and 2008, respectively.

Name of the lakes	Mean depth (m)	Tem. (°C)		Salinity (%)		pH		Chl-a ($\mu\text{g L}^{-1}$)		TOC (mg L^{-1})		Sampling time (day/month)	
		Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Lake Kiba	1.6	26.7	3.5	0.05	0.05	8.6	6.1	44.3	12.0	5.1	4.3	30/7/	8/1
Lake Shibayama	2.2	28.4	4.1	0.06	0.06	8.8	9.4	35.1	3.3	3.9	3.5	22/9	24/2
Lake Kahoku	2.0	27.0	4.8	0.09	0.11	7.9	9.0	57.6	13.7	4.1	4.0	23/9	23/2
Lake Kita	2.1	26.8	4.6	0.75	2.04	8.5	8.2	54.4	46.4	8.8	2.8	30/7	24/1
Lake Mikata	1.3	24.5	2.5	0.6	0.72	9.3	9.4	53.1	13.1	4.2	6.5	20/7	26/1
Lake Suga	no data	24.9	4.7	4.2	3.82	8.2	9.1	6.0	87.7	5.2	5.4	20/7	26/1
Lake Suigetsu	19.0	25.2	5.3	1.32	4.12	8.7	8.9	80.1	82.3	6.3	4.4	20/7	26/1
Lake Kugushi	1.8	25.8	6.6	2.8	22.39	6.7	8.0	23.1	3.0	6.5	4.8	20/7	26/1
Lake Hiruga	14.3	24.2	10.2	13.36	30.1	7.8	8.5	4.8	0.8	2.8	1.4	20/7	26/1
Lake Nojiri	20.8	26.4	2.6	0.04	0.04	8.9	9.7	3.3	1.8	4.9	0.8	23/8	11/2
Lake Suwa	4.1	26.5	1.3	0.08	0.09	8.8	8.2	29.7	3.5	3.7	3.2	23/8	11/2
Lake Kizaki	17.9	25.5	2.8	0.02	0.02	10.1	8.4	16.2	0.9	2.4	3.7	23/8	11/2
Lake Nakatsuna	5.7	24.9	3.0	0.02	0.02	9.4	9.1	4.3	1.5	4.2	2.4	23/8	11/2
Lake Aoki	29.0	22.6	5.0	0.02	0.02	8.9	9.0	4.8	1.3	4.0	1.5	23/8	11/2
Lake Biwa (North)	43.0	22.5	8.4	0.06	0.06	9.3	6.5	5.9	1.6	3.7	3.4	20/7	26/1
Lake Biwa (South)	4.0	29.2	7.4	0.08	0.12	10.1	8.4	7.9	3.0	4.0	5.2	21/9	25/1
Lake Yogo	7.4	24.8	5.4	0.05	0.05	9.3	6.8	11.7	5.0	2.8	1.6	20/7	26/1
Lake Nishino	1.5	25.3	3.9	0.12	0.13	6.8	8.5	7.0	12.4	3.7	7.6	21/9	26/1



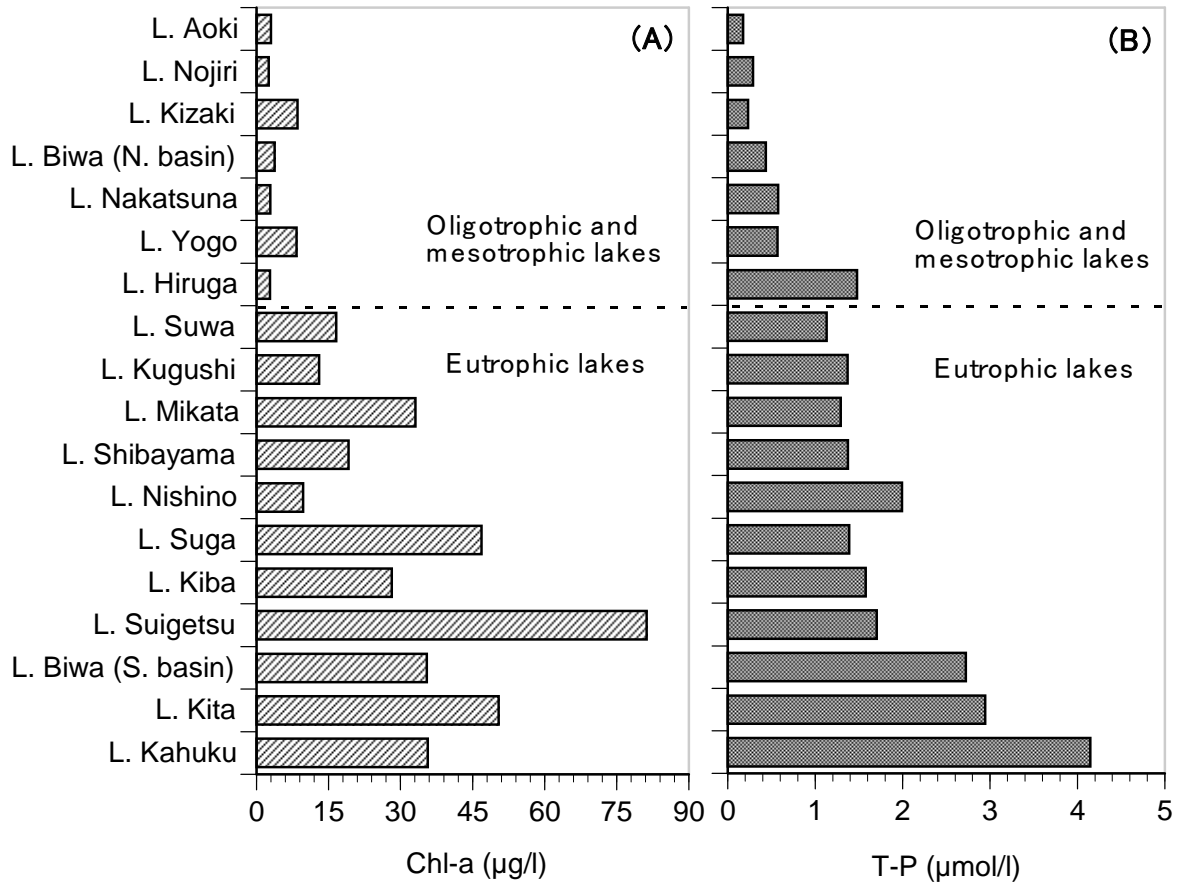
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Fig. 1: Water samples were collected from 18 lakes around Ishikawa, Nagano, Fukui, and Shiga prefectures in Japan.



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Fig. 2: Classification of lakes using mean total phosphate (T-PO₄) and mean total chlorophyll-a (T-Chl-a) concentrations (OECD, 1982).



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 3 Fig. 3: Chlorophyll-*a* (Chl-*a*) and phosphate (PO₄) concentrations in surface water of mesotrophic
 4 and eutrophic lakes (*n* = 1-10). Chlorophyll-*a* (A) and total phosphate (T-P) (B).

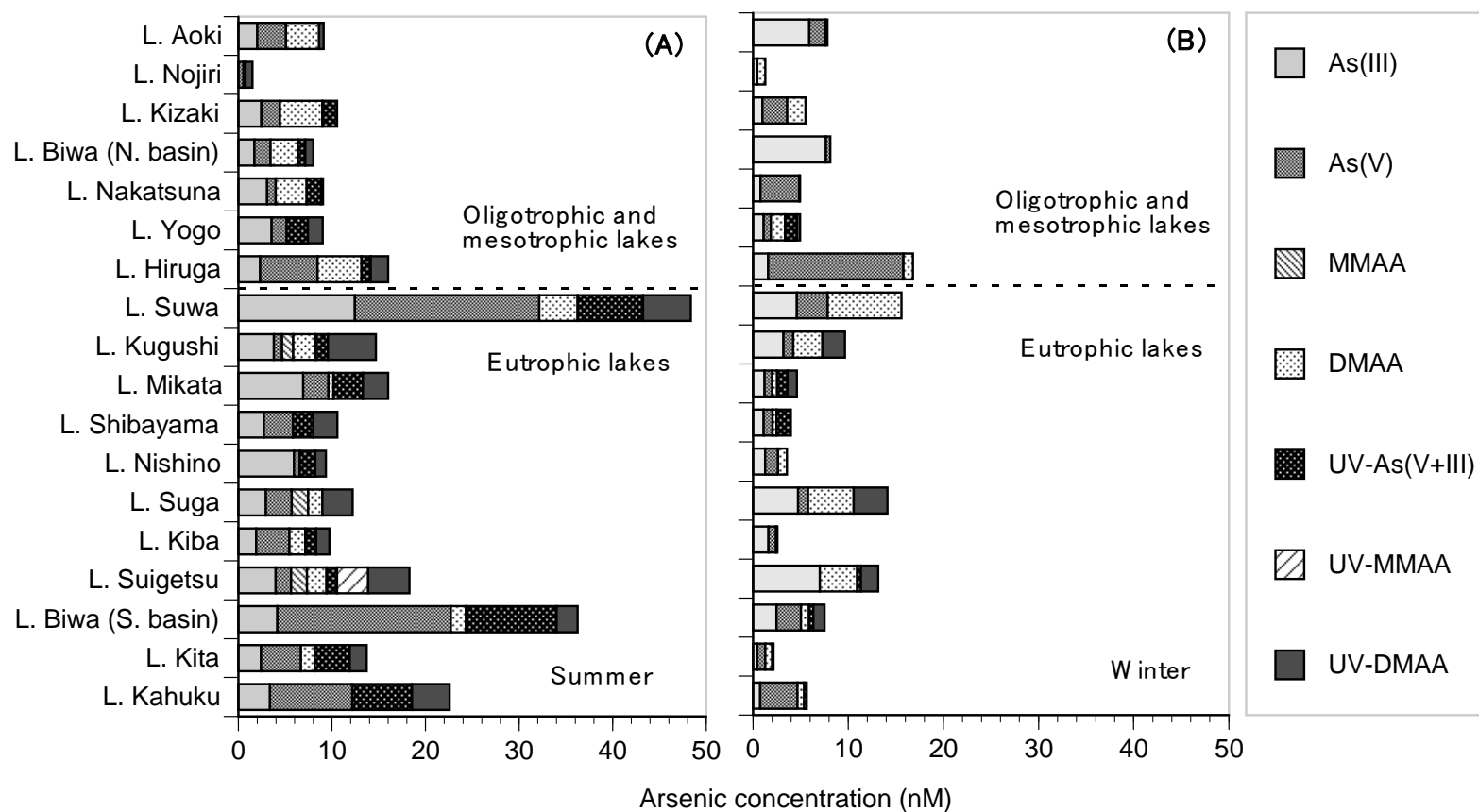


Fig. 4: Mean concentration and distribution of arsenic species in lake waters ($n = 1-10$). The charts show the average data of the 7 oligotrophic and mesotrophic lakes and the 11 eutrophic lakes in Japan.

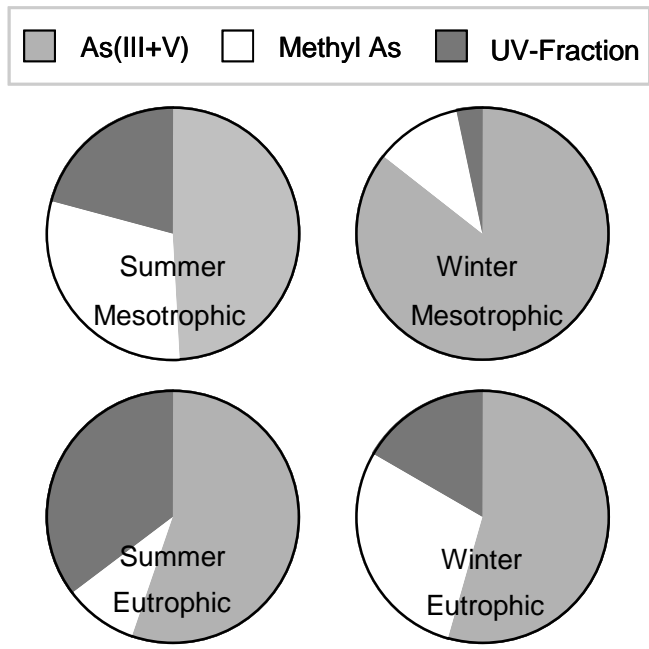
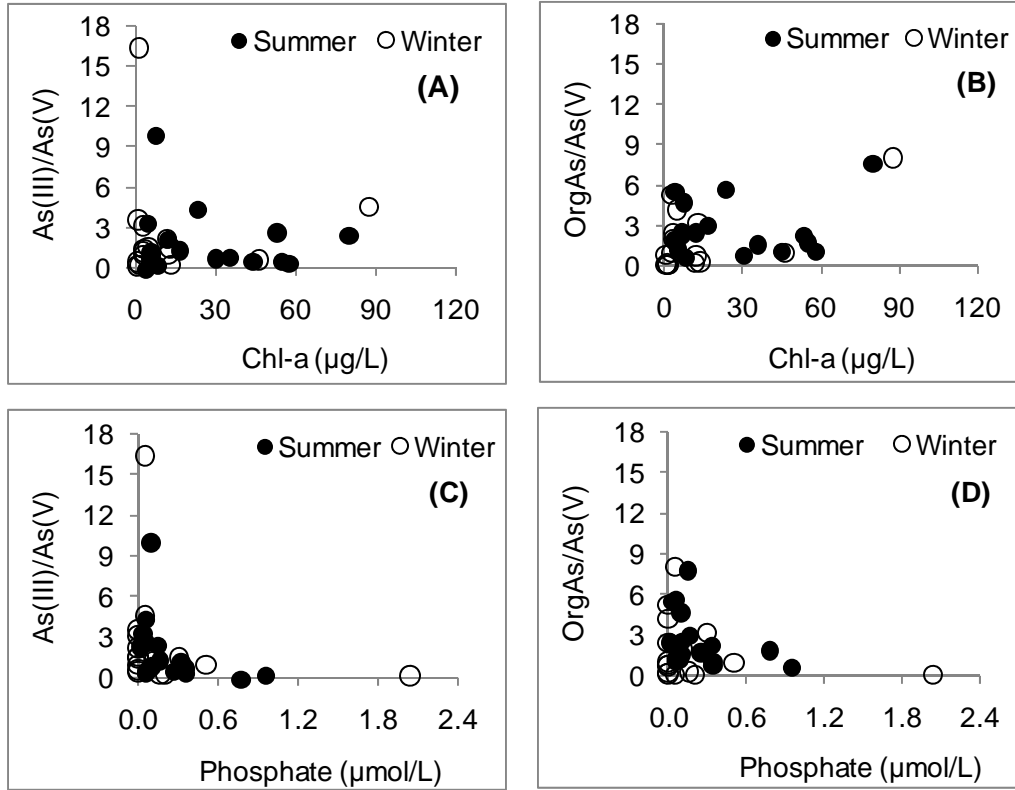


Fig. 5: Ratio of inorganic arsenic, methyl arsenic and the UV-fractions of organic and inorganic arsenic during summer and winter season. The charts show the average data of 7 mesotrophic and 11 eutrophic lakes in Japan ($n = 1-10$).

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24 Fig. 6: Correlation between chlorophyll-a (Chl-a) and the ratio of As(III) or organoarsenicals

25 (OrgAs) to As(V); and between phosphate (PO_4) and the ratio of As(III) or OrgAs to

26 As(V) concentrations in lake waters during summer and winter.

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