Arsenic Speciation and Bioavailability to Macroalgae in Seawater

Dissertation Abstract

Arsenic Speciation and Bioavailability to Macroalgae in Seawater

Graduate School of Natural Science and Technology

Kanazawa University

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ABSTRACT

The metalloid arsenic (As) has no well-known biological function, but it is a widely distributed food chain contaminants and ranked a number one of the top priority hazardous substances in the environment. Arsenate (As(V)) and arsenite (As(III)) are the primary bioavailable inorganic forms in aquatic systems and are actively subjected to biotransform upon their exposure. Marine microalgae and macroalgae have shown enormous As accumulation and transformation capacity, and are the chief contributor of reduced, methylated, and/or other organic As species in seawater, hence play a critical link between cycling of As in the water column and other marine organisms. The recent discoveries of more than 50 arsenicals in marine organisms have extended the research field on As speciation. The information on the formation processes of As species, as well as their nature and distribution, is essential because of their complex chemistry and variable ecotoxicological effects on the marine ecosystems. As detoxification and/or biotransformation processes by the algae are habitat and species-specific, and it is necessary to investigate how marine macroalgae species interact, accumulate, detoxify, and produce As species in seawater and redistributed in marine food web with respect to their importance in As cycling. A series of laboratory culture experiments were designed with different macroalgal species namely *Undaria pinnatifida*, *Sargassum horneri*, *Sargassum patens*, and *Pyropia yezoensis* in seawater, and the following issues have been reported: (a) examination of the bioavailability of As species in terms of algal growth and photosynthetic activity; (b) observation of the accumulation, biotransformation, and extrusion behavior of As under different molar ratios of As and P; (c) investigation of the formation of Fe-plaque with or without coexisting Fe in the algal culture system; (d) demonstration of the modelling of As uptake rate; and (e) elucidation and comparison of the tolerance and metabolism diversity among macroalgae. The results of the proposed experiments are helpful in the understanding of the roles of macroalgae on As biogeochemical cycle in the marine environment.

EXTENDED DESCRIPTION

Introduction:

Algae detoxify As by employing different biotransformation mechanisms either outside or inside of cells including oxidation, reduction, methylation, demethylation, and complex formation for resisting the adverse effect of As. These processes are essential in controlling the mobilization and subsequent distribution of arsenicals in the ecosystem. The extensive research and review works have been carried out in laboratory culture of microalgae (both freshwater and marine) species and focused on the contribution of these organisms in the speciation, toxicity, and metabolism of different As species. There are insufficient researches, and a limited number of laboratory-based culture studies exist with regard to macroalgae but have only started to be studied in the context of As metabolism.

Materials and Methods:

The algae were collected from different locations in the Japan Sea and maintained in the laboratory. The algae were incubated with varying molar ratios of As/P in the laboratory culture system either in the growth chamber or incubator. The different parameters, along with their measurement, is summarized below.

Growth rate	Using the growth rate equation
Chlorophyll fluorescence	Using Pulse Amplitude Modulated (PAM) Fluorimeter
As speciation analysis in culture media	Cold Trap Hydride Generation Atomic Absorption Spectrophotometer (CT-HG-AAS)
Total As analysis	Inductively Coupled Plasma Mass Spectrometry (ICP-MS) followed by Microwave Digestion
Nutrient (P, Fe) concentration in tissues and culture media	Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-MS)
Uptake kinetics	By using the Michaelis-Menten equation
Surface complexation	Washing extraction of TiCE and CBE

Results and Discussion:

The results of the different experiments have been summarized on the following heading:

Arsenic speciation and biotransformation by the marine macroalga *Undaria pinnatifida* in seawater: A culture medium study

Freshwater and marine organisms are capable of metabolizing arsenic (As) efficiently and regulating the As biogeochemical cycles. In this study, *Undaria pinnatifida* was exposed to As(V) (0, 0.1, and 1 μM) and phosphate (P; 1 and 10 μM) in seawater under laboratory-controlled conditions for up to seven days to analyze As biotransformation. The growth and chlorophyll fluorescence of the alga were unaffected by As stress, and statistically insignificant differences were observed among the cultures ($p > 0.05$). As(V) was readily accumulated by this macroalga through phosphate transporters, transformed intracellularly, and excreted into the medium, depending on the As(V) to P molar ratios. The concentration of As(V) and biotransformed species As(III) and DMAA(V) varied significantly in the algal cultures on the basis of the exposure period ($p < 0.05$). The concentration of As(III) was initially higher but decreased with the incubation period, whereas the concentration of DMAA(V) increased gradually. At the end of the incubation, 0.04 and 0.32 μM DMAA(V) were recorded in the media containing 0.1 and 1 μM As(V) with a constant 1.0 μM P, respectively. The results also indicated that the cellular uptake of As(V) and subsequent release of DMAA(V) were inhibited by P in the medium. The biotransformation was consistent with the As(V) detoxification mechanism based on reduction and methylation, which was enhanced by the lower As(V) to P molar ratios. These findings can be helpful in understanding the contribution of macroalgae to As biogeochemistry in marine environments and the potential risks of As dietary uptake.

Comparative biotransformation and detoxification potential of arsenic by three macroalgae species in seawater: Evidence from laboratory culture studies

Algae accumulate and metabolize arsenic (As) and facilitate cycling and speciation of As in seawater. The laboratory-controlled macroalgal cultures were exposed to different molar ratios of As(V) and phosphate (P) in seawater for evaluating the uptake and metabolism of As, as a function of As(V) detoxification through biotransformation. Chlorophyll fluorescence of algal species was not significantly affected by the culture conditions ($p > 0.05$).

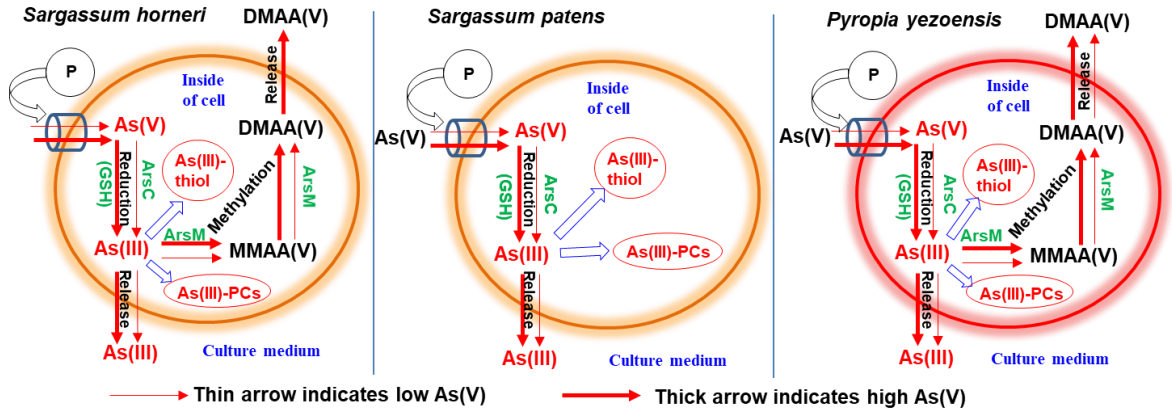

Figure 1: Schematic diagram showing the biotransformation potential of three different macroalgae species under various molar ratios of As/P.

Addition of 10 µM P positively reduce As stress, but different As(V)/P ratios significantly affect the growth rate ($p < 0.05$). Algae readily accumulated As(V) after the inoculation, transformed intracellularly, and released gradually into the medium along the incubation period, depending on As(V)/P molar ratios. Reduction and methylation were the leading processes of As(V) metabolism by *Pyropia yezoensis*, whereas *Sargassum patens* showed only the reduction. *Sargassum horneri* reduced As(V) under low level (0.1 µM), but both reduction and methylation were observed under a high level (1 µM). At the end of incubation, 0.17, 0.15, 0.1 µM of reduced metabolite (As(III)) were recorded from 1 µM of As(V)/P containing cultures of *Sargassum horneri*, *Sargassum patens*, and *Pyropia*

yezoensis, respectively. On the other hand, 0.024 and 0.28 µM of methylated metabolite (DMAA(V)) were detected under the same culture conditions from Sargassum horneri and Pyropia yezoensis, respectively. The results also indicated that P in medium inhibits the intracellular uptake of As(V) and subsequent extrusion of biotransformed metabolites into the medium. These findings can help to understand the metabolic diversity of macroalgae species on As biogeochemistry in the marine environment.

Bioaccumulation and biotransformation of arsenic by the brown macroalga Sargassum patens C. Agardh in seawater: effects of phosphate and iron ions

The toxicity and bioaccumulation and biotransformation potential of inorganic arsenic (IAs) species As(V) and As(III) were investigated using *Sargassum patens* under laboratory culture for seven days. Algal chlorophyll fluorescence decreased with increasing As(V) and As(III) concentrations, being significantly affected by As(III) treatments. Higher As(III) concentration negatively affected growth rate, and P and Fe limitation greatly enhanced IAs toxicity.

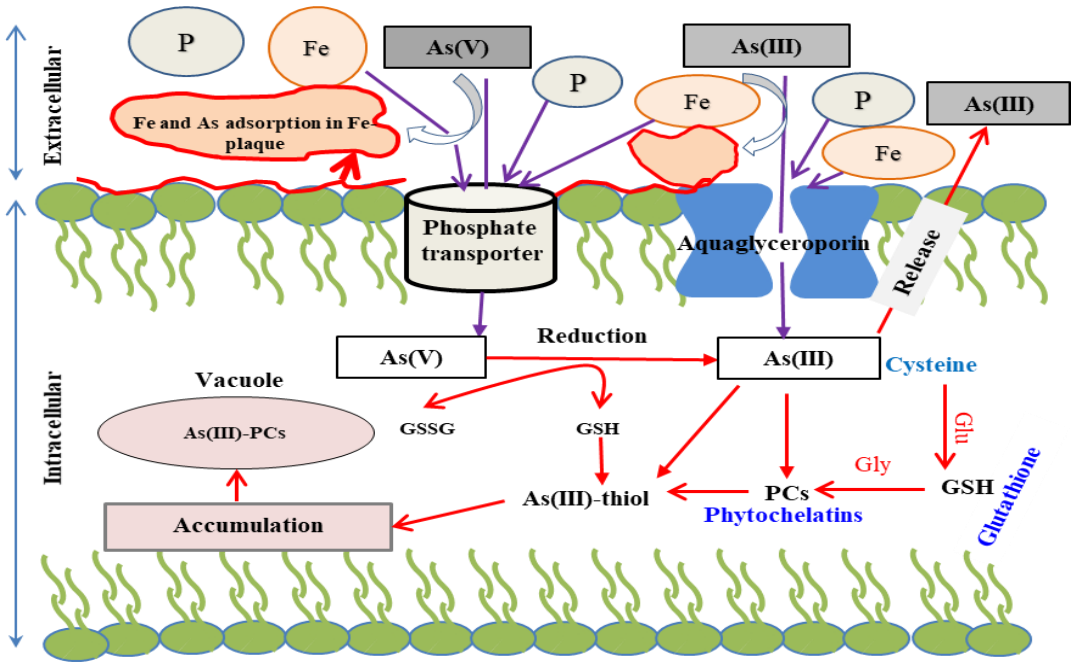

Figure 2: Schematic diagram showing the processes of As detoxification by macroalgae in laboratory culture system

The extracellular, intracellular, and total bioaccumulation of As(III) and As(V) varied significantly depending on initial concentrations and addition of P and Fe. P and Fe availability suppressed intracellular As accumulation in As(V) medium but not in As(III) medium. In P-rich (10 μM) medium, intracellular As was reduced by 4.7% and 9.9% when As(V) in the medium was constant (4.0 μM), under Fe-limited (0 μM) and Fe-rich (10 μM) conditions, respectively. However, the Fe-rich condition positively affected extracellular As accumulation from both As source. Extracellular As increased by 43.5% and 38.8% in P-limited + Fe-rich cultures with 4.0 μM of As(V) and As(III), respectively. Algae exhibited greater absorption and adsorption to As(V) than to As(III). The reduced metabolites of As(III) (3.5 to 4.9% of the total As) and oxidized metabolites of As(V) (2.0 to 3.7% of the total As) were recorded as biotransformed species from coexisting media containing As(V) and As(III) at a constant 4.0 μM, respectively. Both P and Fe had significant influences on the variation in behaviors of IAs. This information is vital in terms of As research in marine ecosystems.

令和元年７月 30 日

学位論文審査報告書（甲）

１．学位論文題目（外国語の場合は和訳を付けること。）

Arsenic Speciation and Bioavailability to Macroalgae in Seawater

（ヒ素のスペシエーションと海洋大型藻類に対する生物可用性）

２．論文提出者　(1) 所　　属　物質化学　専攻

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３．審査結果の要旨（600～650 字）

提出学位論文について、各審査委員が個別に審査した後、令和元年７月 16 日に予備審査会を実施した。令和元年７月 30 日に行われた口頭発表会に引き続き、審査員による審査委員会を開催し、以下のとおり判定した。

σ結合性有機金属化合物は、水俣病の原因となった有機水銀や海洋生物に生殖異常を引き起こす有機スズに代表されるように人類や生態系へダメージを与える場合があるが、自然サイクル中で水生植物の一次生産によっても生成する。本論文では、複数の海洋大型藻類（海藻）について室内培養を実施し、ヒ素化学種の取り込みや生体内濃縮に関与する化学因子とともに、無機ヒ素化学種からメチルヒ素化学種への変換過程を明らかにした。一連の研究において、ヒ素の化学形態別分析法を駆使して、海洋大型藻類を巡る無機およびメチルヒ素化学種の挙動を定量的に解析し、海水中のヒ素に対する化学変換作用は藻類種において多様性を示すことを明らかにした。また、細胞内への５価無機ヒ素の取り込みに関して化学的性質が類似するリン酸イオンと拮抗すること、メチルヒ素の生体内への吸収量は無機ヒ素と比べて著しく低く、成長阻害効果も低いことを見出した。

以上、本研究は、海洋大型藻類のヒ素取り込み挙動や化学種変換作用に現れる多様性を初めて明らかにしたものであり、環境化学分野においてσ結合性有機金属化学種の成因解明に貢献することが期待される。従って、本論文は博士（学術）の学位に値するものと判断する。

４．審査結果　(1) 判　　定（いずれかに○印）　○合　格　・　不合格

(2) 授与学位　博　士（学術）