別記様式第３号－１

学 位 論 文 の 要 旨

氏　　名	澤井　淳
学位論文題目	質量分析による下水中の生態毒性物質の探索に関する研究

本論文では、下水中に存在する有機性の生態毒性物質を網羅的な化学分析により探索する技術を検討し、これまで報告例が見当たらない毒性物質などを発見した。

第 1 章は、現在、欧米諸国で実施されている排水等の毒性削減および毒性同定の手法について整理し、既存の手法の課題を明確にした。また、有機物質が原因となるケースにおいて、既存の手法を補完するため、網羅的な質量分析技術が必要であることを述べた。さらに、様々な分野において活用されている網羅的な質量分析技術の活用事例を整理し、生態毒性試験、網羅的な質量分析および多変量解析を組み合わせる手法の新規性、適用可能性について述べた。

第 2 章は、負イオン化モードでのノンターゲットスクリーニング分析による未知物質の探索・同定能力を実証した。下水処理水に既知の毒性物質 5 物質を添加して模擬試料を調製し、その藻類生長阻害試験結果、高分解能 LC/MS を用いた分析結果を得た。得られた結果に対して多変量解析を適用した結果、試料の毒性強度が試料中のトリクロサンのピーク面積により説明可能とのモデルが構築され、毒性物質の添加条件と一致した。以上により、負イオン化モードでのノンターゲットスクリーニング分析の有効性を確認した。

第 3 章は、第 2 章で対象としなかった正イオン化モードで測定可能な有機物質の探索・同定能力を実証した。正イオン化モードでは、1 つの物質に対して複数種類のアルカリ金属類の付加イオンが検出されやすく、解析が困難と予想される。下水処理水に既知の毒性物質 5 物質を添加して模擬試料を調製し、その藻類生長阻害試験結果、高分解能 LC/MS

を用いた分析結果を得た。多変量解析により模擬試料中の毒性物質（添加した物質）を推定した結果、同族体が11物質発見され、界面活性剤の混合物と推定された。11物質のうちピーク面積が最大の物質の分子式は$C_{14}H_{31}NO$と推定され、データベースマッチングしたところ、*N*,*N*-ジメチルドデシルアミン=*N*-オキシド（DDAO）と推定され、毒性物質の添加条件と一致した。以上により、ノンターゲットスクリーニング分析結果および多変量解析を用いた網羅的探索が正負の両イオン化モードで可能なことを実証した。

第4章は、設置地域、処理水量などが異なる17か所の下水処理場から採取した流入下水を対象に、藻類生長阻害試験およびGC/EI/QMSを用いたターゲットスクリーニング分析を実施した。本分析における同定は、電子イオン化（EI）により得られるマススペクトルおよびリテンションインデックスの一致に基づいて行われた。さらに、予め取得された各物質の検量線情報に基づいた定量分析が行われた。その結果、すべての流入下水の藻類生長阻害を90%以上説明可能な物質が発見され、2種類の界面活性剤であった。これらのうち1物質については、下水からの検出例やリスク評価事例を発見することができなかった。

第5章は、第2章および第3章で有効性を確認した技術を、5か所の小規模下水処理場の流入下水に適用した。その結果、各試料の藻類生長阻害率とピアソンの相関係数0.9以上で相関関係が認められる5物質を発見した。このうち1物質を対象に、精密質量および安定同位体パターンから分子式を推定し、さらに衝突誘起解離により得られたプロダクトイオンから構造を推定した。推定結果に基づいて標準物質とのコクロマトグラフィーを実施したところ、両性界面活性剤であると同定できた。本物質の藻類生長阻害試験を実施したところ、阻害活性が認められた。本物質が下水から検出された例および藻類生長阻害の検討例は発見することができなかった。

第6章は、本研究で得られた結果を総括した。

別記様式第４号

Summary of Doctoral Dissertation

Title of Doctoral Dissertation:

Mass Spectrometric Exploration of Organic Ecotoxicants in Sewage

Name: Atsushi Sawai

In this study, a technique for exploring organic ecotoxicants in sewage water was developed by coupling liquid chromatography-high resolution mass spectrometry (LC/HRMS) with multivariable analysis (MVA). With the developed technique, an unreported ecotoxicant in actual sewage-treatment-plant (STP) influents was successfully identified.

Chapter 1 reviewed the three conventional methods to explore ecotoxicants: toxicity reduction evaluation (TRE), toxicity identification evaluation (TIE), and effect-directed analysis (EDA). They were developed in the early 1980s and implemented in Western countries. TRE/TIE utilized gas chromatography/low resolution mass spectrometry (GC/LRMS) to explore ecotoxicants, so their applications to sewage water containing complex matrices have difficulties by nature. EDA also utilized only GC/LRMS in its early stage of development, however, a recent modification to the method adopted LC/HRMS as well and it became possible to detect a great number of candidate ions. Although significant ions among them must be determined to explore major ecotoxicants in samples, such a methodology has not yet been established. Thus, the objective of this study was to develop a technique for exploring organic ecotoxicants in sewage water by coupling LC/HRMS with MVA.

Chapter 2 demonstrated the applicability of a non-targeted mass spectrometric analysis in a negative ionization mode. From three different STPs, three effluent samples were collected, and five known ecotoxicants were added to the samples to simulate toxic STP effluent samples. The non-targeted screening of the simulated samples was conducted using LC/HRMS. Whole effluent toxicity (WET) tests were conducted by using *Raphidocelis subcapitata* as the test organism. Relationships between the peak volumes of detected components and the samples' toxicities were analyzed by orthogonal projections to latent structure analysis-regression analysis (OPLS-RA), correlation analysis, and multiple regression analysis. An ion of *m*/*z* 286.9432 was found as a possible monoisotopic ion of one of the added toxicants. Its molecular formula was determined by its accurate mass and natural isotopic pattern. Searching the determined molecular formula in an advanced mass spectral database suggested that the found toxicant was triclosan, an antibacterial and antifungal agent. It was identified as triclosan by a co-chromatography using a reference standard reagent. Because triclosan was the dominant toxicant among the added five, the applicability of the developed method was successfully demonstrated.

Chapter 3 demonstrated the applicability of a non-targeted mass spectrometric analysis in a positive ionization mode that was not examined in Chapter 2; an analyte ionized in this mode is likely to produce several adduct ions of alkali metals, and therefore exploration of unknown toxicants will be formidable. From three different STPs, three effluent samples were collected, and three known ecotoxicants were added to simulate toxic STP effluent samples. The non-targeted screening of the simulated samples was conducted using LC/HRMS. Relationships between the peak volumes of detected components and the samples' toxicities were analyzed by OPLS-RA, correlation analysis and multiple regression analysis. These analyses suggested that the major ecotoxicant was a surfactant because its eleven homologues had been detected in the samples. Structural elucidation of a representative ion of the homologues

was conducted by database matching and it was identified to be *N*,*N*-dimethyldodecylamine=*N*-oxide (DDAO). The toxicity contributions of DDAO to the simulated samples were outstanding compared with the other added ecotoxicants. Thus, we concluded that the applicability of the developed method in the positive ionization mode was successfully demonstrated.

Chapter 4 explored significantly ecotoxic compounds in the STP influents collected from 17 different STPs; it was performed with a targeted screening by using GC/LRMS. In this screening, both a result of column retention time matching and that of mass spectrum matching were used as the criteria to identify a compound. Identified ecotoxicants were tentatively quantified by GC/LRMS and their ecological risks were subsequently examined. Two surfactants showed apparent risks, hence their concentrations were further determined to confirm them. Algal growth inhibition tests of the STP influents were conducted using *Raphidocelis subcapitata* as the test organism. More than 90% of the inhibition of each sample was accounted for by the determined two surfactants concentrations. This is the first repot of the surfactant detection in sewage waters to the best of our knowledge.

Chapter 5 explored an unknown ecotoxic compound in STP influents collected from five different STPs; it was performed with the non-targeted screening developed in Chapters 2 and 3. The screening revealed five candidates whose Pearson's correlation coefficients were equal to or greater than 0.9. The structural elucidation of one of the candidates with multistage mass spectrometry suggested that it was an amphoteric surfactant. Co-chromatography was conducted with reference chemical reagent of the amphoteric surfactant. The candidate's column retention time in an actual STP sample the reference standard reagent were in good agreement with each other, and the same could be said for its mass spectra. Therefore, it was successfully identified as the amphoteric surfactant. The maximum concentration of the amphoteric surfactant in these STP influents was determined as 0.0391 mg/L. The algal growth inhibition test of the amphoteric surfactant was performed because such a study has not yet been published; its 72h-NOEC was determined as 3.9 mg/L. Its margin of exposure (MOE) was calculated to be 100, indicating that further ecotoxicological risk assessment is needed.

Chapter 6 summarized the results of this study.