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学位論文題目 **Analyses of prokaryotic communities in Lakes Nyos and Monoun (Cameroon) and bacteriological assessment of groundwater quality in the vicinities of both lakes**

（カメルーン国のニオス湖及びマヌーン湖における原核生物群集の解析と両湖周辺における地下水の細菌学的水質評価）

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ABSTRACT

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Lakes Nyos and Monoun intriguing ecosystems have been monitored for about the last three decades following their CO₂ gas explosions. Despite the important role that prokaryotes play in lakes' nutrient cycles, their community composition have not been investigated in both lakes. Equally, the microbial quality of groundwater, that is a very important commodity for people living in the vicinities of both lakes have not been assessed heretofore. The lack of knowledge regarding the two latter fields weakens the understanding of the lakes' biogeochemistry and the awareness of the general issues related to water quality.

This work consists of three parts. Parts one and two focus on the study of bacterial and archaeal communities composition of Lakes Nyos (I) and Monoun (II). The study used Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE), a profiling technique, as well as cloning/sequencing of 16S rRNA genes, on both lakes samples. Furthermore, quantitative analysis (qPCR) was performed on Lake Monoun's samples to assess the prokaryotic abundance. The results of prokaryotes community studies on both lakes revealed that bacterial and archaeal communities are distributed along the water column following a stratified pattern in both lakes, with a rich microbial diversity likely to include several novel microorganisms. Physico-chemical data and microbial sequences suggested a close correspondence of the potential microbial functions to the physico-chemical pattern of the lakes. However, it appeared that prokaryotic communities markedly differed between both Lakes.

Community members in Lake Nyos were represented by the potential bacterial phyla Actinobacteria (28.3%), Firmicutes (19.6%), Proteobacteria (15.2%), Candidate divisions (TM7, OP8, DO1) (10.9%), Bacteroidetes (8.7%), Chlorobi and Caldiserica (6.5% each) and Fusobacteria and Cyanobacteria (2.2% each). The phyla Thaumacheota was the only (100%) potential representant of archaeal communities.

For Lake Monoun, the retrieved sequences were distributed into six phyla for bacteria. Sequences matching with bacteria of the Proteobacteria group dominated (63.7%), followed by Chloroflexi and Spirochaetes (12.1% each), then Firmicutes (6.0%), and finally Bacteroidetes (3.0%) and Nitrospirae (3.0%). As for sequences obtained with the archaeal primer, they were distributed into the phyla Euryarchaeota

(23.3%) and Thaumarchaeota (20.0%). The highest (56.7%) proportion did not match with known archaea and was termed as unclassified. Absolute quantitative PCR of the 16S rRNA gene for bacteria and archaea showed that bacteria were numerically more important than archaea in all the samples and that bacterial abundance decreases while archaeal abundance increases with depth.

In the third part (III), the study focused on identifying biological indicators of pollution in groundwater sources in the vicinities of Lakes Nyos and Monoun. Culture methods were used to detect and count total coliforms, fecal coliforms and fecal streptococci in water samples. Results revealed the presence of the three bacterial indicators in nearly all the water sources. In the Lake Monoun area, all the 19 analysed samples contained total coliforms. Then, 16 (84.2%) and 17 (89.5%) samples contained faecal coliforms and faecal streptococci, respectively. All the 17 samples collected around Lake Nyos contained total and faecal coliforms, and 16 (94.1%) samples contained faecal streptococci. The high bacterial counts raise a concern on the suitability of those water sources which are used for drinking and other domestic purposes. This study provides evidence of fecal contamination in most of the water sources in both areas, implying health threat to consumers and suggesting the necessity to improve attention to environmental sanitation control.

The work presented in this thesis is a first approach in studying the microbial diversity in Lakes Nyos and Monoun and in assessing bacterial quality of groundwater in the vicinities of both lakes. It brings light on two important aspects, namely: on one hand, the knowledge of community composition and diversity, which increase our understanding of the plausible biogeochemical functioning of both lakes, and which can be used for monitoring and predicting environmental change and guide for further studies in microbial ecology. On the other hand, the detected bacterial indicators give a snapshot of the status of the water resource in the vicinities of the lakes. Which can be used as a guide to water management, and to predicting the presence of certain pathogens, and therefore, protecting local people's health.

【論文審査の結果の要旨】

当学位審査委員会は当該学位論文を詳細に査読し、かつ平成 27 年 7 月 22 日（水）に博士論文研究の公聴会を実施し、同日、論文内容の最終審査と最終試験を実施した。以下に審査結果を要約する。

カメルーン国のニオス湖とマヌーン湖では、人や家畜の大量死を引き起こした二酸化炭素のガス爆発が生じてから約 30 年間、二酸化炭素濃度を主とした地球化学的な長期モニタリングがなされている。しかし、この間、生物学的なモニタリング等の研究は全くされて来なかった。なかでも、細菌と古細菌から構成される原核生物は、湖の栄養塩の循環において大変重要な役割を果たしているにもかかわらず、両湖水中の原核生物そのものやそれらの群集構造についても、いまだに研究されていない。また、両湖の近隣の住民にとって生活に必需である地下水の微生物学的水質評価もなされていない。そこで、本研究において、申請者はこれらの基盤となる研究に着手した。

まず、ニオス湖とマヌーン湖における細菌と古細菌の群集構造に関する研究は、両湖の試料水について、Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE)を行った。また、原核生物の存在量を評価するために、マヌーン湖の試料水について定量 PCR を行った。そして、両湖の原核生物の群集構造を調べた結果から、両湖の成層構造に応じて細菌と古細菌が分布していることを示唆した。さらに、数多くの新規性の高い微生物を含むことや、微生物種の多様性に富んでいることを明らかにした結果から、地球物理化学的パターンと微生物種の潜在的機能とが密に対応していることを推定した。興味深いことに、ニオス湖では、Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Chlorobi, Caldiserica, Fusobacteria, Cyanobacteria の各門の細菌と、Thaumarchaeota 門のみの古細菌が検出された一方、マヌーン湖では、Proteobacteria, Chloroflexi, Spirochaetes, Firmicutes, Bacteroidetes, Nitrospirae の各門の細菌と、Euryarchaeota と Thaumarchaeota の各門の古細菌が検出されたように、両湖水中の原核生物の群集構造が大きく異なっていることを見出した。また、細菌あるいは古細菌の 16S rRNA 遺伝子を標的とした定量 PCR から、全ての試料で細菌は古細菌より多く、水深が深くなるにつれて細菌は減少し、古細菌は逆に増加する傾向を示した。

ニオス湖とマヌーン湖周辺の地下水源の汚染を生物学的指標によって調査する研究では、全大腸菌群、糞便性大腸菌群、糞便性連鎖球菌を計測するための培養法が用いられた。その結果、ほとんどすべての水試料においてこれら 3 種の指標細菌が存在することを見出した。マヌーン湖周辺で採取した 19 の試料水では、全大腸菌群をすべての試料水から検出した。そして、糞便性大腸菌群を 16 試料水(84.2%)、糞便性連鎖球菌を 17 試料水(89.5%)から検出した。ニオス湖周辺で採取した 17 の試料水では、全大腸菌群と糞便性大腸菌群をすべての試料水から、また、糞便性連鎖球菌を 16 試料水(94.1%)から検出した。このような指標細菌の高い検出率は、両湖周辺の地下水を飲料水や他の家庭生活用水として使用することへの懸念を引き起こす。そして、本研究結果から、両地域のほとんどの水源は糞便汚染されていて

消費者の健康を脅かす恐れがあることが考えられ、地域における公衆衛生への意識改革が必要であることを示唆した。

本博士論文研究は、ニオス湖とマヌーン湖における微生物学的多様性とその周辺地下水の細菌学的水質の評価に関する最初の取り組みである。その結果、両湖における微生物の群集構造と多様性についての知見が得られ、両湖の生物学的、地球化学的機能に関する我々の理解を深めるとともに、両湖の今後の微生物生態学に関する研究において、大きな指針になるものと思われた。一方、両湖周辺の地下水の糞便汚染状況を評価できた研究として、本地域における水管理や住民の健康管理を目指すための基盤となることが強く伺えた。

本博士論文研究の成果は、申請者が筆頭著者となって査読付き国際科学雑誌へ2編公表されており、関連する国際会議やシンポジウムにおいて発表されている。

以上により、当該審査委員会は本申請論文が博士（理学）の学位を授与するに値するものと認め、合格と判定した。

なお、本研究は、JICA-JST の「地球規模課題対応国際科学技術協力（SATREPS）：カメルーン火山湖ガス災害防止の総合対策と人材育成」（プロジェクトリーダー：東海大学大場武教授）プロジェクトの一つとして、カメルーンからの留学生である申請者が富山大学で行ったものである。また、これらの多大な成果は、SATREPS プロジェクトの1つの目的である人材育成の成果ともなる。

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