様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月 １２ 日現在

機関番号：１５２０１
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研究課題名（和文）The study of nanoplastic transformation in the aquatic environment and their transformations of polycyclic aromatic hydrocarbons

研究課題名（英文）The study of nanoplastic transformation in the aquatic environment and their transformations of polycyclic aromatic hydrocarbons

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：研究の焦点は環境中プラスチックの変化にありました。大気と海洋環境で、プラスチック表面の酸化と結晶化がより進行しました。ナノ・マイクロプラスチックに対する有機物コロナの影響が実験室研究で調査されました。有機物は酵母細胞壁へのナノプラスチックの付着を妨げた。しかし、有機物はミジンコに対するプラスチックの毒性を軽減しませんでした。バイオフィルムの分析により、プラスチック表面で増殖する微生物は周囲の水中の微生物とは異なることが示されました。これは他の有機汚染物質が変換される程度に影響を与えます。 例えば、PAH のより有害な同族体への酸化は、水生環境における微生物活動と相関していることが判明しました。

研究成果の学術的意義や社会的意義
Like many contaminants, plastics are likely more harmful to organisms after they have been transformed in the environment. This research explored this transformation by investigating various types of change, from polymer organization to microbial community structure on plastic surfaces.

研究成果の概要（英文）：The focus of the study was on how plastics are changed in the environment. In both the atmospheric and marine environment, plastic degradation resulted in a greater degree of oxidation and crystallization of plastic surfaces. The influence of organic matter coronas on nanoplastics and microplastics was investigated in laboratory studies. Using yeast as a model organism, it was found that organic matter prevented the adherence of nanoplastics to the cell wall, likely because of the destabilization of the nanoplastic colloid. However, organic matter did not alleviate the toxicity of microplastics to Daphnia. Analyses of biofilms indicate that the microbes that grow on plastic surfaces differ from the microbes in the surrounding water. This may affect the extent to which other organic contaminants are transformed. For example, it was found that the oxidation of PAHs into more harmful congeners was correlated with microbial activity in the aquatic environment.

研究分野：Environmental pollution

キーワード：Microplastics　Nanoplastics　Raman spectroscopy　FTIR　PAHs　Environmental pollution　polymer crystallinity　carbonyl index

Outline of research achievements (300 characters Japanese). Also, need 1000 characters in English (for webpage)

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Scientific and social significance of your research achievements (300 characters)

Like many contaminants, plastics are likely more harmful to organisms after they have been transformed in the environment. This research explored this transformation by investigating various types of change, from polymer organization to microbial community structure on plastic surfaces.

Keywords: microplastics, nanoplastics, Raman spectroscopy, FTIR, hydroxylated PAHs, ecotoxicology

1. 研究開始当初の背景(background)

Like other contaminants, plastics change once released into the environment. However, in contrast to organic contaminants, plastics have a much different fate because they are solid surfaces. In the environment, plastic surfaces are subject to oxidation, polymer chain reorganization and modification by the formation of corona films, whether by organic matter or by microbes. This is part of the process by which they fragment into microplastics and nanoplastics. This will also affect the extent to which contaminants are adsorbed to the surface. While plastics are known to change, there is not much known about this, particularly in the context of the Japanese environment.

2. 研究の目的

In this context, the purpose of the research then was to explore how these changes may be occurring in the environment. There are various aspects to this that were specifically investigated. One major component was to provide some assessment of the how much microplastic exists in the environment and in what state they exist in. The second was to understand how these smaller plastics (microplastics and nanoplastics) interact with the surrounding environment, in particular with natural organic matter and microbes. Finally, the extent to which PAHs may be sequestered/metabolized in the presence of these microbes and plastics was explored, given that one of the major concerns with regards to plastic pollution is that they are substrates for other contaminants to adsorb and potentially even transform.

3. 研究の方法

a. Environmental microplastics studies: These were conducted primarily through field work and analysis of samples using Raman spectroscopy and FTIR. Samples were taken from various media across Japan, including air, water, sand and organisms (i.e. amphipods).
b. Corona studies: The effect of the organic matter corona on nanoplastics was studied specifically using Raman imaging and the heteroaggregation was studied with a combination of atomic force microscopy and dynamic light scattering. Yeast cells were used for the study of nanoplastic imaging studies, while Daphnia were used for toxicology studies (LC_{50} and reproduction tests). In addition, the microbial corona community structure on environmental plastics was analyzed through DNA sequencing.
c. OHPAH analyses: Methods were first developed for the chromatographic separation of hydroxylated PAH congeners using a combination of HPLC-fluorescence and LC-MS. Analyses were done on environmental samples to assess the extent to which these actually exist in the environment.

4. 研究の成果

a. Environmental microplastics: Various surveys have been performed on environmental samples and indicate that in general, plastics that exist in the environment exist in a crystalline state. In particular, atmospheric microplastics are degraded compared to those found in the water (i.e. Figure 1 shows the difference in polystyrene crystallinity). It is likely that the microplastics found in the environment are the result of surface level degradation of plastics that shed and enter the environment. Further microplastics were found in coastal amphipods,

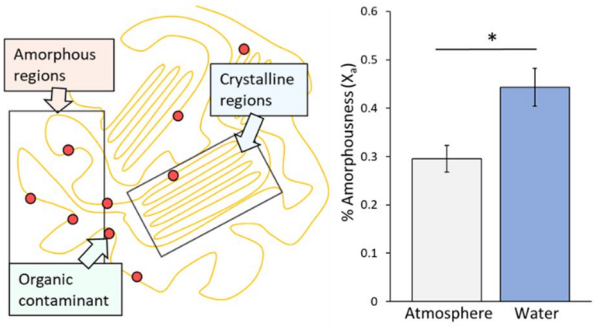

図1. *Polymer organization can be loose (amorphous) or more densely packed (crystalline). Plastics in the air were more crystalline* than those in water

with similar signs of degradation. Beached plastics also showed signs of oxidation, based on FTIR analyses, but also increased crystallization. Based on these results, it is likely that the microplastics that are found in the environment are from the crystallinity induced fragmentation and provide insight into how microplastics are introduced into the environment.

b. The effect of the organic matter corona on nanoplastic fate: The role of organic matter with nanoplastics was studied using yeast as a model organism and Raman imaging to monitor the fate of various types of functionalized nanoplastics. As shown in Figure 2 while nanoplastics are liable to adhere to the outer cell wall of an organism, with the addition of humic acid, this is prevented. It is likely that the humic acid is destabilizing the nanoplastic colloid, causing it to be removed from solution. This was corroborated with the AFM analysis. The removal of nanoplastics from the solution will have consequences for the toxicity of the particle, as it may be the case that in the environment, organic matter (i.e. humic acid) protects cells from nanoplastic exposure, though if the nanoplastics are removed from solution, it implies that organisms in the sediment are more likely to be exposed to nanoplastics.

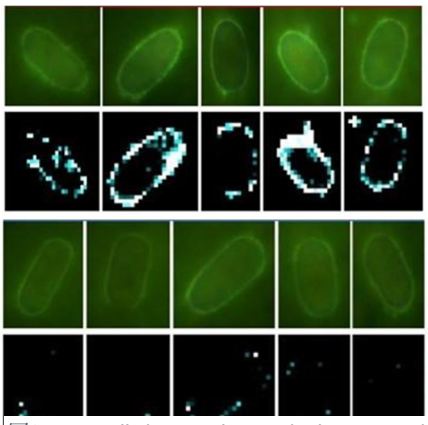

図2. Yeast cells (top row in green) when exposed *to polystyrene nanoplastics accumulate particles* around the cell wall (second row). However, these are absent when humic acid is included. *(bottom row).*

c. Characterization of the ecocorona: Microbes that colonize plastic surfaces are seen to be different from those in the surrounding aquatic environment as was observed with samples taken from the Japan Sea (Figure 3). The plastic surface then is a substrate upon which a discrete microbial colony forms. This has implications for the toxicity of the particle. For example, one species found in abundance are vibrio spp. which are known to cause illness. Similar laboratory experiments using Winowgradskly columns were made with various types of plastics, but did not show indication that biofilms formed during the 8 month incubation period. This suggests that under anaerobic environments, the types of colony formation observed in open waters would not be observed in anaerobic sediments and therefore plastic degradation would be less likely in those environments.

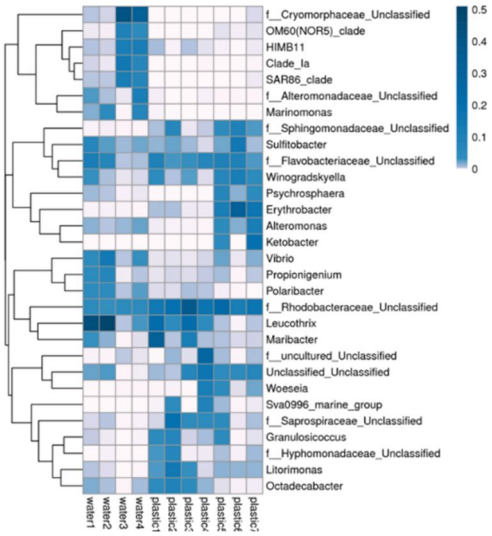

図3. *The relative abundance of the identified microbes differ between plastics (plastics 1-7)* and the surrounding water (water 1-4).

d. Hydroxylated PAH analyses: Both laboratory and environmental analyses were performed for PAH metabolism. Environmental analyses were taken in the Hiroshima region and increases in PAH oxidation to their hydroxylated congeners was correlated with ATP concentrations, suggesting that OHPAH formation is dependent on microbial activity in the environment. Further experiments were conducted in the laboratory to examine whether algae could be responsible for the conversion of PAHs into their oxidized congeners. Test examining the protective effect of these complexes from PAH pollution on daphnia did not indicate that there was any particular effect.

５．主な発表論文等

〔雑誌論文〕　計8件（うち査読付論文　8件／うち国際共著　8件／うちオープンアクセス　0件）

１．著者名 Suzuki Nobuo, Honda Masato, Sato Masayuki, Yoshitake Shuhei, Kawabe Kimi, Tabuchi Yoshiaki, Omote Toshiki, Sekiguchi Toshio, Furusawa Yukihiro, Toriba Akira, Tang Ning, Shimasaki Yohei, Nagato Edward G.... Hayakawa Kazuichi	４．巻 234
２．論文標題 Hydroxylated benzo[c]phenanthrene metabolites cause osteoblast apoptosis and skeletal abnormalities in fish	５．発行年 2022年
３．雑誌名 Ecotoxicology and Environmental Safety	６．最初と最後の頁 113401～113401
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.ecoenv.2022.113401	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Hayakawa Kazuichi、Tang Ning、Matsuki Atsushi、Inomata Yayoi、Toriba Akira、Nagato Edward G.	４．巻 280
２．論文標題 Calculating source contributions to urban atmospheric polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons using 1-nitropyrene and pyrene: An application to an Asian dust event	５．発行年 2021年
３．雑誌名 Chemosphere	６．最初と最後の頁 130662～130662
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.chemosphere.2021.130662	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Masato Honda, Koki Mukai, Edward Nagato, Seiichi Uno, Yuji Oshima	４．巻 18
２．論文標題 Correlation between Polycyclic Aromatic Hydrocarbons in Wharf Roach (Ligia spp.) and Environmental Components of the Intertidal and Supralittoral Zone along the Japanese Coast	５．発行年 2021年
３．雑誌名 International Journal of Environmental Research and Public Health	６．最初と最後の頁 630
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ijerph18020630	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Kazuichi Hayakawa, Ning Tang, Akira Toriba, Edward G Nagato	４．巻 265
２．論文標題 Calculating sources of combustion-derived particulates using 1-nitropyrene and pyrene as markers	５．発行年 2020年
３．雑誌名 Environmental Pollution	６．最初と最後の頁 114730
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.envpol.2020.114730	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Kazuichi Hayakawa, Ning Tang, Atsushi MAtsuki, Yayoi Inomata, Akira Toriba, Edward G Nagato	4．巻 280
2．論文標題 Calculating source contributions to urban atmospheric polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons using 1-nitropyrene and pyrene: An application to an Asian dust event	5．発行年 2021年
3．雑誌名 Chemosphere	6．最初と最後の頁 130662
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.chemosphere.2021.130662	査読の有無 有
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1．著者名 Nagato, E.G., Hayakawa, K.	4．巻 250
2．論文標題 The presence of nitroarenes formed by secondary atmospheric processes in the Japanese freshwater environment	5．発行年 2019年
3．雑誌名 Environmental Pollution	6．最初と最後の頁 554-558
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.envpol.2019.04.049	査読の有無 有
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1．著者名 Hayakawa, K., Tang, N., Toriba, A., Nagato, E.G.	4．巻 -
2．論文標題 Calculating sources of combustion-derived particulates using 1-nitropyrene and pyrene as markers	5．発行年 2020年
3．雑誌名 Environmental Pollution	6．最初と最後の頁 -
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1．著者名 Katte Yasuharu、Saito Junya、Nagato Edward G.	4．巻 30
2．論文標題 Abundance and characterization of microplastics in amphipods from the Japanese coastal environment	5．発行年 2023年
3．雑誌名 Environmental Science and Pollution Research	6．最初と最後の頁 35505～35512
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s11356-023-25878-w	査読の有無 有
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〔学会発表〕　計3件（うち招待講演　2件／うち国際学会　0件）

1．発表者名 長門豪
2．発表標題 The transformation of microplastics in the Japan Sea
3．学会等名 The overview of Microplastic Pollution and the Vision of Ecoscience (online)（招待講演）
4．発表年 2021年

1．発表者名 Edward Gou Nagato
2．発表標題 The changing nature of microplasticsin the aquatic environment
3．学会等名 Joint International Symposium on Sustainable Development and Environmental issues（招待講演）
4．発表年 2019年

1．発表者名 長門豪Edward
2．発表標題 The Deposition of Nitroarenes in the Japanese Freshwater Environment
3．学会等名 第25回中国四国支部分析化学若手セミナー
4．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関