Form 3

Annual Report

LaMer, Ehime University

Date (27/02/2017)

To Director of LaMer

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Affiliation: National Institute of Animal Health-NARO

Position: Principal

Researcher Name in print

1. Project / Meeting title

Nationwide survey of emerging micro pollutants in aquatic environment in Sri Lanka: distributions and effects

2. Members of project / meeting

Name	Affiliation	Position	Contribution part
PI Keerthi S. Guruge	National Institute of Animal Health-NARO	Principal Researcher	Sample collection, Extraction, Data analysis, Report
LaMer Faculty member in charge Takahashi Shin	Ehime University, Division of Environmental Chemistry and Ecotoxicology	Associated Professor	Screening of pollutants by automated identification and quantification system (AIQS-DB)
Nomiyama Kei	Ehime University, Division of Environmental Chemistry and Ecotoxicology	Associated Professor	Quantitative analysis of selected pharmaceuticals and personal care products (PPCPs)

Annual Report 2016

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1. Title: Nationwide survey of emerging micro pollutants in aquatic environment in Sri Lanka: distributions and effects [スリランカにおける新規微量汚染物質の国内広域調査：その分布と影響]

2. Members' names and affiliations:

Name	Affiliation	Position
Guruge Keerthi S.	National Institute of Animal Health-NARO	Principal Researcher
Takahashi Shin	Ehime University, Division of Environmental Chemistry and Ecotoxicology	Associated Professor
Nomiyama Kei	Ehime University, Division of Environmental Chemistry and Ecotoxicology	Associated Professor

3. Aim: Over recent years, human health concerns related to the occurrence of pollutants in aquatic environments in Sri Lanka have been greatly increased. However, no extensive monitoring studies were found for micro pollutants, including persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), flame retardants, pesticides, pharmaceuticals and/or other emerging contaminants. Therefore, this study was carried out to gather information of pollutant levels in the aquatic ecosystems in Sri Lanka.

4. Procedure:

4.1. Sampling

Water samples (n=13, approx.1L) were collected in March 2013 from several environmental settings which included lakes, streams, a waste water canal and a coastal lagoon (Fig.1) in Sri Lanka. Aliquot of water samples (500mL) were extracted soon after the collection by solid phase extraction (SPE) method using pre-conditioned Empore C18 disks. The C18 disks were allowed to dry for ~30min under vacuum and transported to Japan. Then eluted with ethyl acetate, dichloromethane (DCM) and a mixture of Ethyl acetate/DCM (1:1) of 5 mL, 5 mL and 10mL, respectively (EPA method 525.2). The extracted were combined and evaporated to near dry under a gentle stream of nitrogen at 35℃ and dissolved in 200μL of toluene.

About 150mL water samples in polypropylene bottles were transported to CEMS-Ehime

University for PPCPs analysis.

4.2. Screening of pollutants by automated identification and quantification system (AIQS-DB).

Three selected samples (procedure blank, MW4 and GL1) were analyzed by AIQS-DB system (Shimadzu Technical report, 2013). In brief, analysis using the AIQS-DB is based on pre-registered database comprising retention times, mass information, and calibration curves for 942 chemicals with 8 standards. The samples were analyzed using a quadrupole GC-MS (Shimadzu GCMS-QP2010 ultra) equipped with a J&W DB-5ms column. The Performance evaluation of GC/MS system is conducted by using n-Alkane Standards (C9-C33). Sample were added a mixture of internal standards (10 mg/L) containing 8 kinds of deuterium-labeled polycyclic aromatic hydrocarbons before injection. Target compounds were determined by Total Ion Monitoring (TIM) in positive mode of ionization. Briefly, peak identification and quantification of targets were based on the 80% similarity to the standards in the data base, S/N >3 and at least three times higher their blank levels.

4.3. Quantitative analysis of selected pharmaceuticals and personal care products (PPCPs)

A 50mL of filtered water sample was spiked with internal standards, loaded onto a preconditioned Oasis HLB Plus Light cartridge. The analytes retained in the cartridge were eluted with methanol/MTBE mixture, and the eluate was concentrated to 0.2 mL under N2 flow. The residue was reconstituted in methanol/Milli-Q water and filtered through a cellulose membrane syringe filter. Ninety-four PPCPs in water samples were analyzed by a UPLC- Qtrap 5500 mass spectrometer operating in electrospray ionization (ESI) positive and negative modes with multiple reaction monitoring (MRM).

5. Result

5.1 AIQS-DB analysis.

The concentrations of semi-quantitatively measured chemicals in the water samples from Sri Lanka are given in Table 1. Twelve chemicals were detected in MW4, which was a stream in the rural tea plantation area in Sri Lanka, ranged from 3.2 to 1066 ng/L. In contrast, 17 chemicals were detected in GL1, which was an urban sewage cannel, located in southern Sri Lanka, ranged from 24 to 7234 ng/L. Cholesterol was found to be the main pollutant at the MW4 while stigmasterol was detected at the highest level at GL1. The concentrations of plant and human/animal sterols and caffeine at GL1 were several times higher than those at MW4 indicating greater human activities in the former

location. The levels of contaminants detected in this study suggest that urban surface waters may be highly polluted compared to rural waterways, however, further studies are essential to identify pollution hot spots in Sri Lanka.

Nevertheless, detection of human fecal matter biomarkers such as cholestanol and coprostanol at both locations suggested that these waterways were contaminated with human feces. Fecal contamination may release antimicrobial-resistant bacteria (ARB) into the aquatic environment, which is a growing world-wide public health threat in terms of the dissemination of superbugs.

Table 1. Chemicals detected in surface waters from Sri Lanka (ng/L)

Chemical	Usage/origin	Concentration	
		MW4	GL1
Naphthalene	PAH, industry		124
1,2-Dichlorobenzene	solvent, industry	3.6	
1,3-Dichlorobenzene	solvent/organic synthesis, industry	3.6	
1,4-Dichlorobenzene	insecticidal fumigant, business/household	3.2	
Bisphenol A	intermediate resin, business/household		209
Dicyclohexyl phthalate	plasticizer, business/household		111
Diethyl phthalate	plasticizer, business/household		586
Di-n-octyl phthalate	plasticizer, business/household	37	
Methyl palmitate	fatty acid methy ester, business/household	22	
Elaidic acid methyl ester	fatty acid methy ester, business/household		664
Oleic acid methyl ester	fatty acid methy ester, business/household		174
Stearic acid methyl ester	fatty acid methy ester, business/household	46	
Benzyl alcohol	cosmetics/fuel additive/solvent/leaching from tire		1720
beta-Sitosterol	plant sterols	848	3224
Cholestanol	human/animal sterols	940	608
Cholesterol	human/animal sterols	1066	3699
Coprostanol	human/animal sterols	864	1432
Stigmasterol	plant sterols	559	7234
2-(Methylthio)-benzothiazol	leaching from tire/ business/household/traffic		24
Caffeine	stimulant, human activity	1002	5081
Bensulide	herbicide agriculture		686
Oxabetrinil	herbicide agriculture		27
Thiobencarb	herbicide agriculture		7.2

5.2 *Distribution of PPCPs in Sri Lankan surface water*

Among the PPCPs analyzed, carbamazepine (antiepileptic agent) and trimethoprim (an

antibiotic) were detected in all locations (Fig. 1) ranging 0.25 to 71.2 ng/L and 0.13 to 42.4 ng/L, respectively. DEET, an insect repellent, was mostly found in populated areas with the highest detection in the SG1, where is one of the best tourist attractions in Sri Lanka. Interestingly, sulfamethoxazole (934 ng/L) and trimethoprim antibiotics were found in high concentrations in the fish farm (No7) suggesting that these chemicals have been used in the ornamental fish farming in Sri Lanka. Other PPCPs such as diclofenac, mefenamic acid, gemfibrozil, losartan, cetirizine, phenytoin, tramadol, clarithromycin and triclocarban concentrations were relatively higher in the two streams from Kandy (K2) and Nuwara Eliya (NE2) suggesting that these compounds can be discharged from nearby hospitals (Fig 2).

6. Other Activity

   The 15th Special Lecture of LaMer Project and a workshop were conducted under the theme of “Measurement of environmental samples by using GC/MS with automated identification and quantification system” on January 31, 2017 at the Miura Memorial Hall, Faculty of Agriculture, Ehime University.

6. Perspectives in future:

   In this study we found that AIQS-DB analysis can be used as a semi-quantitative and inexpensive method to screen large amount of pollutants in the water samples. However, improvement of sample extraction method may be important to obtain accurate measurements for a wide range of pollutants. Thirty-eight out of 72 targeted PPCPs were detected in Sri Lanka. This data suggests that discharge from hospitals and fish farming can be the point sources of several pharmaceuticals pollution in Sri Lankan surface waters.

   For the year 2017, more samples from Sri Lanka will be semi-quantitatively analyzed by AIQS-DB to elucidate distribution of chemicals in aquatic ecosystems. In addition, more specific quantitative analysis for PPCPs in water (hospital wastewater) and biota (i.e. fish) will be carried out to clarify source and distribution of emerging chemicals, and their risk to human and wildlife in Sri Lanka. Moreover, integrated studies of both chemical substances and biological contaminants such as antibiotic resistance genes in Sri Lanka would be interested in future.

7. List of publication / conference presentation”.

   1. Guruge KS. 2017. Occurrence of micro pollutants and their impact on human health in Sri Lanka. 15th Special Lecture of LaMer Project, Faculty of Agriculture, Ehime

University.

Reference.

1. EPA method 525.2. 1995.Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry.
2. Shimadzu Technical Report, 2013. Development of a Novel Automated Identification and Quantification System with a Database for GC-MS. C146-E203.

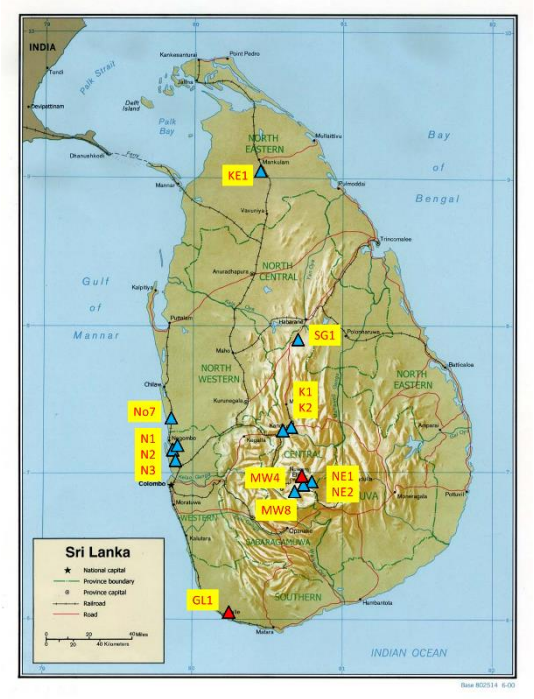

Fig. 1 Sample locations. Samples marked in red (MW4 and GL1) were employed for AIQS-DB analysis while samples from KE1, SG1, K1, K2, NE1, NE2, N1, N2, GL1 and No7 were employed for PPCPs analysis.

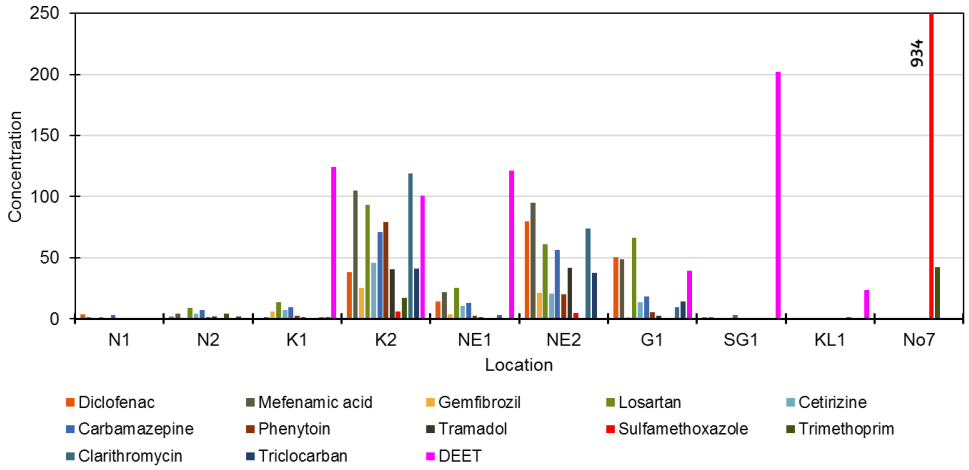

Fig 2. Concentrations (ng/L) of selected PPCPs in Sri Lanka surface waters