Emerging Concern of Micropollutants: Recommended Inclusion of Antibiotics Monitoring in the Environmental Effects Monitoring Program for Municipal Wastewater Effluents

Mofizul Islam and Qiuyan Yuan

***Abstract*—Currently, the micropllutants such as antibiotics are not included in the environmental effects monitoring program for municipal wastewater effluent. This paper presents the importance of the integration of the antibiotics in EEM program. In addition, various types of the sample collection and analysis method for antibiotics monitoring are discussed. It is concluded that antibiotic monitoring program would give an insight view of the effectiveness of antibiotic removal measure of the environmental management and would be very crucial tool for finding the proper remedial actions for the wastewater treatment authorities.**

***Index Terms*—Micropollutant, anitbiotics, environmental effect monitoring, municipal wastewater effluent, aquatic ecosystems.**

I. Introduction

Alteration in aquatic ecosystem caused by human activities (i.e. effluent discharge) is a global concern. Environmental Effects Monitoring (EEM) program in Canada is a scientific process to detect the change in the ecosystem and measure the affected level of contaminants. That helps to protect the ecosystem identifying the effectiveness of the measure of environmental management. The governing parameters, both the biotic and abiotic, of an ecosystem represent the level of healthiness of an ecosystem and EEM uses those parameters to evaluate the impact of human activities on ecosystem health.

There are two EEM programs exists in Canada covering the effluents from pulp and paper and metal mining industries implemented through the two regulations, Pulp and Paper Effluent Regulations (PPER) and Metal Mining Effluent Regulations (MMER) under the Canadian Fisheries Act and these programs could be employed to other type of environmental assessment, both regulatory and non-regulatory [1]. Following the nationwide concern of decades for the protection of the environment and ecosystem, recently Canada has begun to expand its EEM program to cover Municipal Wastewater Effluents (MWWE) discharge into the aquatic environment.

The pulp and paper EEM program, as a regulatory tool, works through the technical elements of fish survey, benthic invertebrate community survey, toxicology, tainting, and tissue analyses for dioxins and furans (Environment Canada, 1998) and additionally sub-lethal toxicity, water and sediment of the aquatic EEM program under the MMER [2], [3] to identify the changes due to the exposure of the contaminants. There are still some more important technical elements left behind which are in the lower priority level for the pulp and paper and metal and mining EEM programs, but those elements could be in the top priority list for the monitoring program of the newly proposed MWWE discharge, especially the occurrence of Emerging Concern of Contaminants (ECC), which are also known as Micropollutants, for instance antibiotics, could be in the top most priority level.

The aim of this paper is: 1) to do a study on antibiotics (a selected group of micropollutants) in the perspective of existing EEM programs; 2) to gather information about the fate and effect of antibiotics in the aquatic system; 3) to assess the importance of antibiotics monitoring in the receiving environment of the municipal effluents discharge; 4) to come up with an aspect of antibiotics monitoring consideration for the EEM program of MWWE discharge, which will give an idea about the way of incorporating new technical elements to the existing system, sample collections and data analysis/interpretation procedure; and 5) to do a critical review with some specific recommendations.

II. Antibiotics in EEM Program — Justification

The endorsement of the Canada-wide strategy for the management of MWWE discharge results the proposal of EEM program for the municipal wastewater management systems. It was less important to consider the potential risk of antibiotics exposure to the environment from the pulp and paper and metal and mining industries effluents as there were no potential source of antibiotics and therefore no provision was needed to include antibiotics monitoring in those two EEM programs under the PPER and MMER. But MWWE discharge is one of the main contributor factors for the occurrence of antibiotics and antibiotic resistance organisms in the receiving watershed and should not be wise to ignore it in the EEM program of MWWE discharge.

Antimicrobial use is one of the main contributors to the development and spread of antimicrobial-resistant organisms and MWWE discharge is one of the most important leading factor for the occurrence of antibiotics and antibiotic resistance genes in the aquatic ecosystem. World antibiotics consumption increased more than 35% from 2000 to 2010 and the quantitative figure in the field of agriculture was at about 63,000 tons in 2010. To meet the demands of a

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The authors are with the University of Manitoba, Winnipeg, MB R3V1L9, Canada (e-mail: islammm6@myumanitoba.ca, qiuyan.yuan@umanitoba.ca).

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projected 8.5 billion world population in 2030 [4], antibiotics are predicted to rise by two-thirds - more than 65% to about 105,000 tons [5] in 2030. In Canada, more than 1.5 million kg of antimicrobial active ingredients were distributed and/or sold for use in livestock and poultry sector in 2013. On the other hand, more than 750 human cases of disease caused by resistant Salmonella bacteria (an antibiotic resistance bacteria) have been reported out of a total of about 3,000 human Salmonella cases in the same year [6].

The residual antibiotics from animal and human use can enter into the environment and aquatic ecosystems via numerous pathways, including (but not limited to) wastewater effluents discharge, livestock and poultry waste discharge, clinic and hospital discharge, discharge from clinical laboratories and the related manufacturing facilities, leaching and runoff from agricultural and human waste exposed land.

Wastewater Stabilization Ponds (WWSPs) or lagoons are widely used sewage treatment options for small communities, almost 60% of small municipalities of under 5000 people are served [7] in Canada. But the corresponding resources for the antibiotics removal efficiencies are so limited. On the other hand, removal of pharmaceuticals from the WWTPs is unsatisfactory not only in the large communities located in the remote areas having limited sophisticated technique but also in the developed cities [8], [9], subject to compound-specific properties and factors like types of treatment processes, antibiotics' physicochemical properties, sludge age, etc. For the improved human and environmental health protection and to protect the aquatic biota, many municipal wastewater treatment process need to be improved in Canada [10] before ultimate discharge to the natural aquatic ecosystem.

Having the stimulating ability of certain biological response, antibiotics are the main active ingredients of many Pharmaceutical and certain aquatic organisms are highly prone to have direct toxicity and have suspected potential risk to the aquatic ecosystem [11], [12].

A wide range of veterinary and human antibiotics having the most frequent detection of erythromycin, lincomycin, sulfamethoxazole, trimethoprim, and tylosin were identified (detected 16 out of 30) in source waters [13] and antibiotic resistance bacterial profiles were also detected (12 antibiotics of eight classes) in the swine lagoon effluent in the southeastern United States [14], [15].

III. Integration of Antibiotics Monitoring in EEM Program

A mechanism or scientific tool is highly needed to monitor alterations in receiving environments over the long term exposure to wastewater effluents from the municipal and agricultural farms, and to address these concerns, EEM program need to be introduced as a requirement under the newly proposed MWWE regulations to generate and provide the essential information and to help identifying the effectiveness of the ongoing environmental and ecological fortification measures.

Antibiotics monitoring would be integrated to the EEM program for the MWWE discharge to the environment by following almost similar procedure of existing two EEM programs for the technical analysis and data interpretation of the identified antibiotic resistance species and resultant toxicity. Sampling designs would be done in the best way possible, for example, choosing an upstream as the reference point and downstream as the MWWE exposure area for the Gradient Approach and Upstream/Downstream statistical analysis to identify the significance difference and distance of the affected area. BACI (Before/After, Control/Impact) analysis would be considered for the optimal design, and Reference Condition approach (RCA) analysis would be applied when no upstream or pre-impairment sampling would be possible. Antibiotics monitoring actions would be associated with both biotic (microorganisms, planktons, fishes, benthic invertebrates, amphibians, reptiles, and so on) and abiotic (water, sediments, suspended solids etc.) and a list according to the prioritized importance would be generated through a preliminary critical assessment based on the mostly affected and highly risk level species and environmental components, those would get highly priority importance in the EEM program..

IV. Antibiotics Monitoring: Sample Collection and Analysis

Occurrence of antibiotics both from urban (hotspots of domestic antibiotics) and animal wastewater (veterinary antibiotics) gradually increasing and has become a challenges for the scientists to develop a monitoring program, which could be introduced in the newly proposed EEM program for the MWWE discharge.

A. Aquatic Organism (Bacteria and Plankton)

Synthesized and chemically modified antibiotics are enormous in the watershed of North America, especially in proximity of effluent exposure area. Many organisms are intrinsically resistance to antibiotics and culturable antibiotic resistance bacteria are pervasive in water column [16]. There is a highly potential risk of structural change of leaf-associated bacterial communities due to the exposure of Enrofloxacin (an antibiotic) and significantly reduction of ammonia-oxidizing bacteria and ammonia-oxidizing archaea, which affect the aquatic environment and lead to cause eutrophication [17]. Sterile tools would be used to collect the sample from the water column at a depth of about 15cm to isolate antibiotics and antibiotic resistant bacteria and microorganisms. The antibiotic resistance bacteria and the prevalence of organism resistance to antibiotics would be measured by the procedure and evaluation method of National Committee for Clinical Laboratory Standards [18].

Photosynthetic aquatic organisms (e.g. Cyanobacterium – Anabaena CPB4337 and green alga – Pseudokirchneriella subcapitata) have highly toxicity effect in response to antibiotics exposure and the presence of particular mixture of certain antibiotics in the aquatic ecosystem may have a potential adverse ecological effect even with the currently obtained concentrations in the water column [19]. Niskin, Ruttner or Van Dorm sampler would be used for the discrete sampling of plankton and Plankton Net and/or Schindler-Patalas trap would be used to trap the photosynthetic organisms and plankton from the receiving environment. Scientific analyses would be performed by using TSQ Quantum LC-MS triple quadrupole, HPLC-diode array liquid chromatography, and LC-MS QqQ to determine the exposure concentration of antibiotics and their mixture [19]. The antibiotics toxicological interactions in binary or multicomponent mixtures would be analyzed using the Combination Index (CI) method.

B. Fish, Amphibians and Reptiles

High level of antibiotic resistance bacteria (mucus-dwelling of fish in dorsal fin area) is identified in a freshwater lake [20]. Antibiotics, which are extensively used in human and veterinary treatment, can accumulate in aquatic species, and results enhancing antibiotic resistance microorganisms in fishes, which lead to antibiotic-resistant infections and potential antagonistic side-effects in humans such as allergies [21], [22]. Samples collection process for the biotic species would be almost similar of the EEM program for pulp and paper and/or metal mining. Seine netting and line fishing for the fish, amphibians and reptile species of different trophic levels (i.e. Yellow Perch and Northern Pike for fish) with some other safety measures would be the best approach. The presence of antibiotics and antibiotic resistance organisms, age, tissue, body mass ratio, liver to mass ratio, and gonad size are the metrics that would be used for analysis.

For the analysis of residual antibiotics in the fish samples, a multi-residue method was established (validated according to the requirements of European Decision 2002/657/EC) by using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/ MS) to identify and determining of 34 antibacterial drugs comprised of 3 aminoglycosides, 9 β-lactams, 9 fluoroquinolones, 3 macrolides, 5 sulfonamides, trimethoprim, and 4 tetracyclines [23]. The same procedure could also be followed for the analysis of the samples collected form amphibians and reptiles..

C. Benthic Macro-invertebrates

Amoxicillin (an antibiotic) disturbed to some extent to the haemocyte parameters of bivalves [24]. Invertebrates live in close proximity to the sediment and the hydrophobic antibiotics remain higher level in the bottom part of the watershed. Benthic macro-invertebrates can accumulate certain poisons in aquatic food chain and antibiotics have more potential risk of toxicity to the benthic macro-invertebrates than to the microorganisms and cannot be allowed of wide exposure [25]. Deployment of colonization traps, core samplers, grab samplers, drift samplers, kick net samplers, and artificial substrates are the best approach to collect the benthic macro-invertebrates. The analysis would include density, diversity, species, and taxon richness based on the occurrence of antibiotics, antibiotic resistance organisms, and toxicity for a clear insight view of adverse impact of antibiotics.

D. Abiotic Monitoring

For the monitoring of antibiotics and their metabolites, samples would be collected both from water column and sediments from the MWWE exposure area and both short term and long term fate and effects would be monitored. Concentration of various antibiotics and their metabolites (abiotic parameters) would be measured starting at the point of MWWE input along a gradient away from the source or the downstream. A combination of grab (for solid phase extraction method) and passive sampling (for o-DGT and POCIS) method will be applied to sample from the water phase.

The antibiotics and antibiotics resistance organisms would be extracted from contaminated water by solid-phase extraction (SPE) column and scientific analysis would be done using High-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS) [26].

Passive water samplers – both Diffusion Gradients in Thin-Films for organics (o-DGT) and Polar Organic Chemical Integrative Sampler (POCIS) would also be deployed to identify the occurrence and to monitor routinely of antibiotics and their metabolites in the contaminated water [27]-[29].

A powerful approach of comparison study among o-DGT, POCIS and grab sampling would be carried out to determine the occurrence, fate, and behavior of antibiotics and their metabolites in the aquatic ecosystem and for the outstanding reliable information as well.

E. Sediments and Suspended Solids

Hydrophobic antibiotics get more easily bind to the suspended solids (e.g. high speed - turbulent flow region) and deposited to the bottom of the waterways result the accumulation of antibiotics in the sediments. Antibiotic resistance genes more stable in soil and higher concentrations have been identified in sediment than in water column [30]. Sediments sampling would be performed with a Ponar Grab Sampler and suspended solids would be extracted from the collected water either by a solid phase extractor or by the filtration process and scientific analysis would be completed using a HPLC–MS/MS.

V. Challenges and Some Recommendations

Antibiotics monitoring in the existing two EEM programs was not very important due to the lack of potential source of antibiotics exposure to the environment from those effluents. But municipal wastewater and livestock discharge are mostly liable for the exposure of antibiotics into the aquatic ecosystem. So, it is very necessary to consider the potential risk of antibiotics in the newly proposed MWWE discharge regulation and to take necessary steps for a specific guideline of EEM program including the residual antibiotics in the aquatic ecosystem.

Due to the trace concentration and diversity, the challenges associated with antibiotics are not only limited to the wastewater treatment process but also belongs to the identification and analysis procedures as well [31].

No specific data or guideline have been found about the maximum allowable threshold limit of different types of antibiotics and their metabolites in the aquatic environment for the healthy living of the aquatic biota and for the safe use of the antibiotic-exposed water. Consideration of antibiotics in the EEM program for the MWWE would create new opportunity and also new challenges to introduce the maximum allowable threshold limit of different kind of antibiotics (at least for those contaminants whose occurrence have been identified mostly in the aquatic environment and which have high risk of toxicity and high potential of adverse xenobiotic effect) in the different types of water body.

Though 60% of small municipalities' wastewater are treated by lagoons in Canada [7], no information have been found regarding the antibiotics and their metabolites removal capacity of this treatment process. In the same way, WWTPs are not well designed to remove those contaminants and also do not have sufficient data of their antibiotics removal efficiencies.

Very limited information is available about the fate and effect of antibiotics in aquatic environment, especially on Canadian rivers and lakes with extreme climatic region having a wide range of temperature changes between summer and winter. Need a vast and detail study about the fate and effect of antibiotics and antibiotic resistance genes in the aquatic biota (fishes, benthic invertebrates, amphibian, reptiles, and so on).

In the Canadian extreme climatic condition, antibiotics stability condition, degradation, catalyzing element, sorption pattern and many other crucial events have not been studied in presence of different fertilizer, pesticides or even the influence of eutrophic elements (nutrients). No data available about the type of antibiotics and their metabolites are getting exposed to the water column form the Canadian MWWE discharge and furthermore study is needed to figure out about each and every types of antibiotics and their all possible metabolites which have a potential adverse effect to aquatic biota either by causing toxicity or creating antibiotic resistance organisms.

VI. Conclusion

Incorporation of antibiotics monitoring in the EEM program for the MWWE discharge, would open up a new horizon to solve many unknown questions and would be the first step to face the emergence of newly concerns about the potential risks of micropollutants in perspective of Canadian aquatic environment. Monitoring the biotic and abiotic components of the contaminants receiving environment under the EEM program for MWWE discharge, would explore the occurrence of antibiotics and antibiotic resistances microorganisms. This program would also give an insight view of the effectiveness of antibiotic removal measure of the environmental management and would be very crucial tool to figure out the remedial actions for the wastewater treatment authorities.

Conflict of Interest

The authors declare no conflict of interest".

Author Contributions

Mofizul Islam conducted the research and wrote the paper; Dr. Qiuyan Yuan edited the paper. All authors had approved the final version.

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Mofizul Islam holds a MSc degree in environmental engineering. He graduated from the Department of Civil Engineering, University of Manitoba. His Master's thesis was titled "Removal of refractory contaminants from mature landfill leachate utilizing fungi and evaluation of lignocellulosic enzymatic activity".

Qiuyan Yuan joined the Department of Civil Engineering, University of Manitoba in July of 2013 as an assistant professor. She earned her PhD degree from the University of Manitoba.

Dr. Yuan has 10 years research and development experience in sustainable nutrient removal and recovery processes applied to wastewater and biosolids. Since 2014, her research area has expanded to solid waste treatment and management.