Release of Antibiotic Resistant Bacteria and Genes in the Effluent and Biosolids of Five Wastewater Utilities in Michigan

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ABSTRACT

The purpose of this study was to quantify the occurence and release of antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) into the environment through the effluent and biosolids of different wastewater treatment utilities including an MBR (Membrane Biological Reactor) utility, conventional utilities (Activated Sludge, Oxidative Ditch and Rotatory Biological Contactors-RBCs) and multiple sludge treatment processes (Dewatering, Gravity Thickening, Anaerobic Digestion and Lime Stabilization). Samples were monitored for tetracycline resistant genes (*tetW* and *tetO*) and sulfonamide resistant gene (*sul-1*) and tetracycline and sulfonamide resistant bacteria.

METHODS

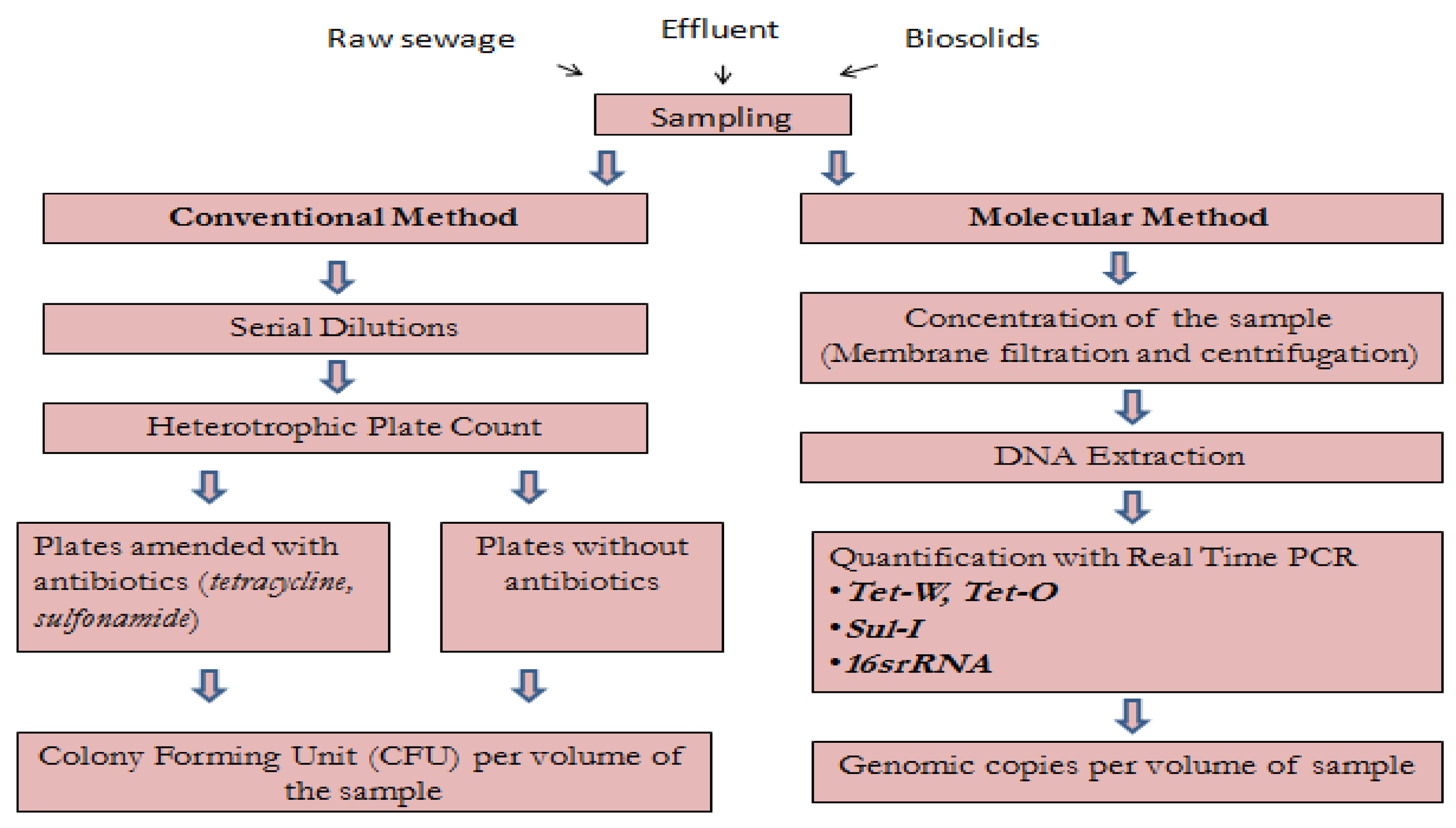

Table 1: Wastewater Treatment Characteristics.

	EAST LANSING	IMLAY	ROMEO	TRAVERSE CITY	LANSING
Wastewater treatment process (Biological treatment)	Activated Sludge (AS)	Oxidation Ditch (OD)	Rotating Biological Contactors (RBCs)	Membrane Biological Reactor (MBR)	Activated Sludge (AS)
Capacity	18.8 MGD	0.9 MGD	2.1 MGD	17.0 MGD	37.0 MGD
Average flow	13.4 MGD	0.4 MGD	0.8 MGD	8.5 MGD	20.0 MGD
Discharge Rate	14.1 MGD	0.02 MGD	0.8 MGD	4.0 MGD	19.0 MGD
Disinfection	*Chlorine (Cl)*	*Ultra-Violet (UV)*	*Chlorine (Cl)*	*Ultra-Violet (UV)*	*Ultra-Violet (UV)*

Table 2: Biosolids Treatment Characteristics.

	EAST LANSING	IMLAY	ROMEO	TRAVERSE CITY	LANSING
Sludge treatment	Dewatering	Gravity Thickening	Anaerobic Digestion	Anaerobic Digestion	Lime Stabilization
Disposal of sludge	Landfill	Agricultural land	Agricultural land	Agricultural land	Agricultural land
Disposal rate (dry tons per year)	3596	118	125	850	4380
% solid	18.05%	1.49%	7.98%	4.85%	9.20%

Table 3: Primers and Probes used in this study.

Target	Primers	Sequences (5'-3')	Annealing temperature (°C)	Amplicon Size (bp)	References
Tet-W	tet(W)-FV tet(W)-RV	GAGAGCCTGCTATATGCCAGC GGGCGTATCCACAATGTTAAC	64	168	Aminov et al 2001
Tet-O	tet(O)-FW tet(O)-RV	ACGGARAGTTTATTGTATACC TGGCGTATCTATAATGTTGAC	60	171	Aminov et al 2001
Sul-I	sul(I)-FW sul(I)-RW	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	55.9	163	Pei et al 2006
Bacteria 16srRNA	BACT1369F PROK1492R TM1389F (Probe)	CGGTGAATACGTTCYCGG GGWTACCTTGTTACGACTT CTTGTACACACCGCCCGTC	56	143	Suzuki et al 2000

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Suzuki, M. T., L. T. Taylor, and E. F. Delong. 2001. Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations via 59-Nuclease Assays. Appl. Environ. Microbiol. **66(11)**:4605-4614.

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RESULTS

ARGs and ARB concentrations in the final effluent were found to be in the range of ND(non-detectable)-2.33×10^6 copies/100mL and 5.00×10^2-6.10×10^5 CFU/100mL respectively. Concentrations of ARGs (*tetW* and *tetO*) and 16s rRNA gene in the MBR effluent were observed to be 1-3 log less, compared to conventional treatment utilities. Significantly higher removals of ARGs and ARB were observed in the MBR facility (range of removal: 2.57 to 7.06 logs) compared to that in conventional treatment plants (range of removal: 2.37-4.56 logs) ($p<0.05$). Disinfection (Chlorination and UV) processes did not contribute in significant reduction of ARGs and ARB ($p>0.05$). In biosolids, ARGs and ARB concentrations were found to be in the range of 5.61×10^6-4.32×10^9 copies/g and 3.17×10^4-1.85×10^9 CFU/g, respectively. Significant differences ($p<0.05$) were observed in concentrations of ARGs (except *tetW*) and ARB between the advanced biosolid treatment methods (i.e., anaerobic digestion and lime stabilization) and the conventional dewatering and gravity thickening methods.

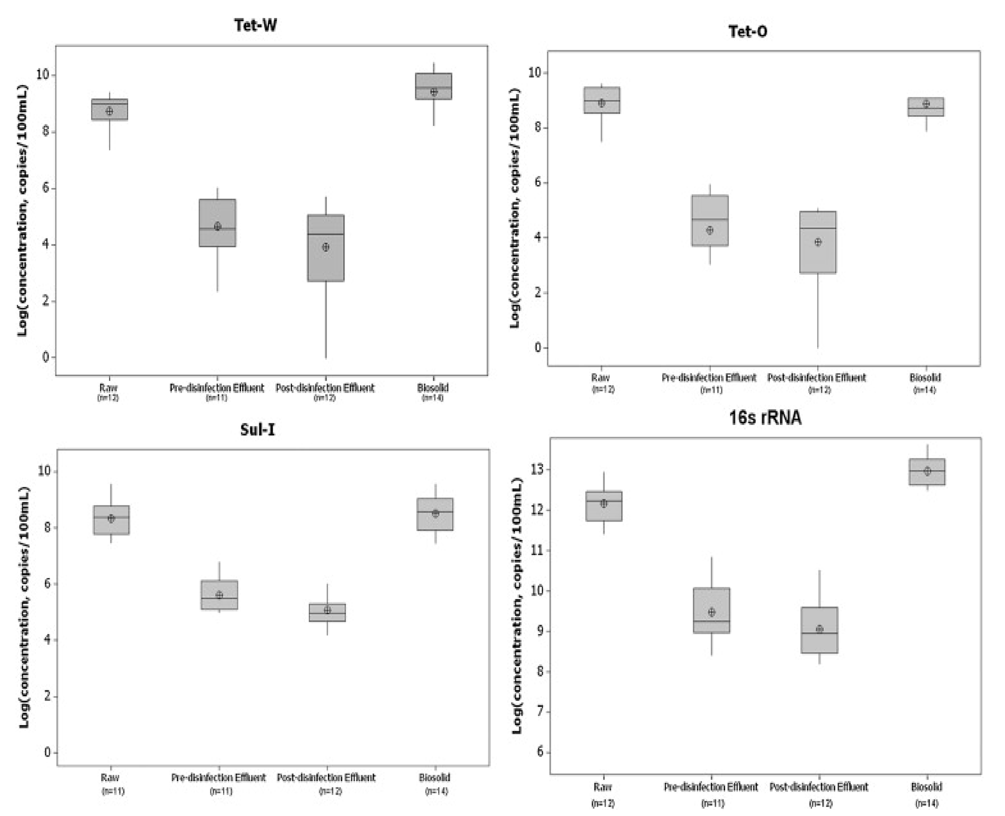

Fig.1 Log concentration (copies/100 mL) of tetracycline resistant genes (*tetW*, *tetO*), sulfonamide resistant gene (*SulI*) and 16s rRNA gene abundance at different sampling points of all the five wastewater utilities.

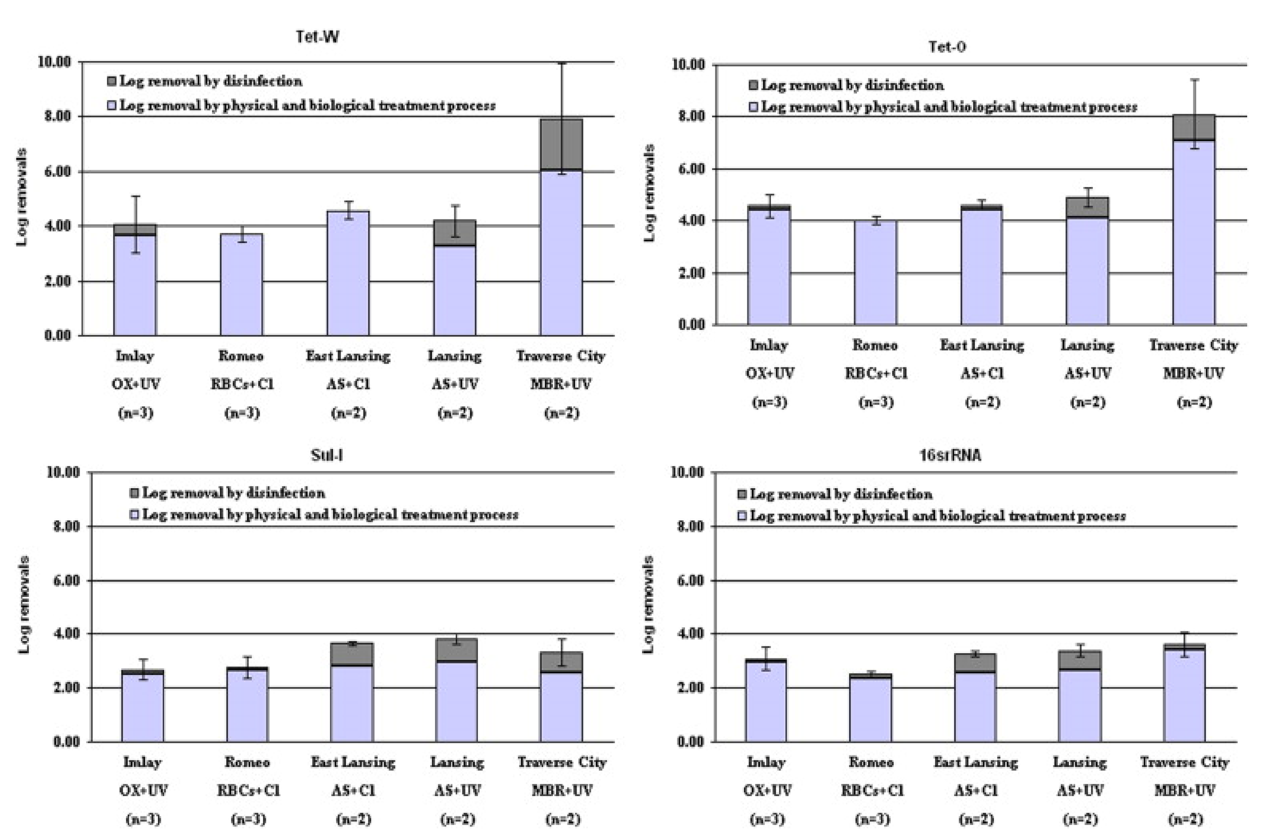

Fig. 2 Log removals of tetracycline resistant gene (*tetW*, *tetO*), sulfonamide resistant gene (*SulI*) and 16s rRNA gene abundance from wastewater sample of different wastewater utilities. Abbreviations: OX = Oxidative ditch; RBCs = Rotatory Biological Contactors; AS = Activated Sludge process; MBR = Membrane Biological Reactors; Cl = Chlorination disinfection; UV = Ultraviolet radiation disinfection;

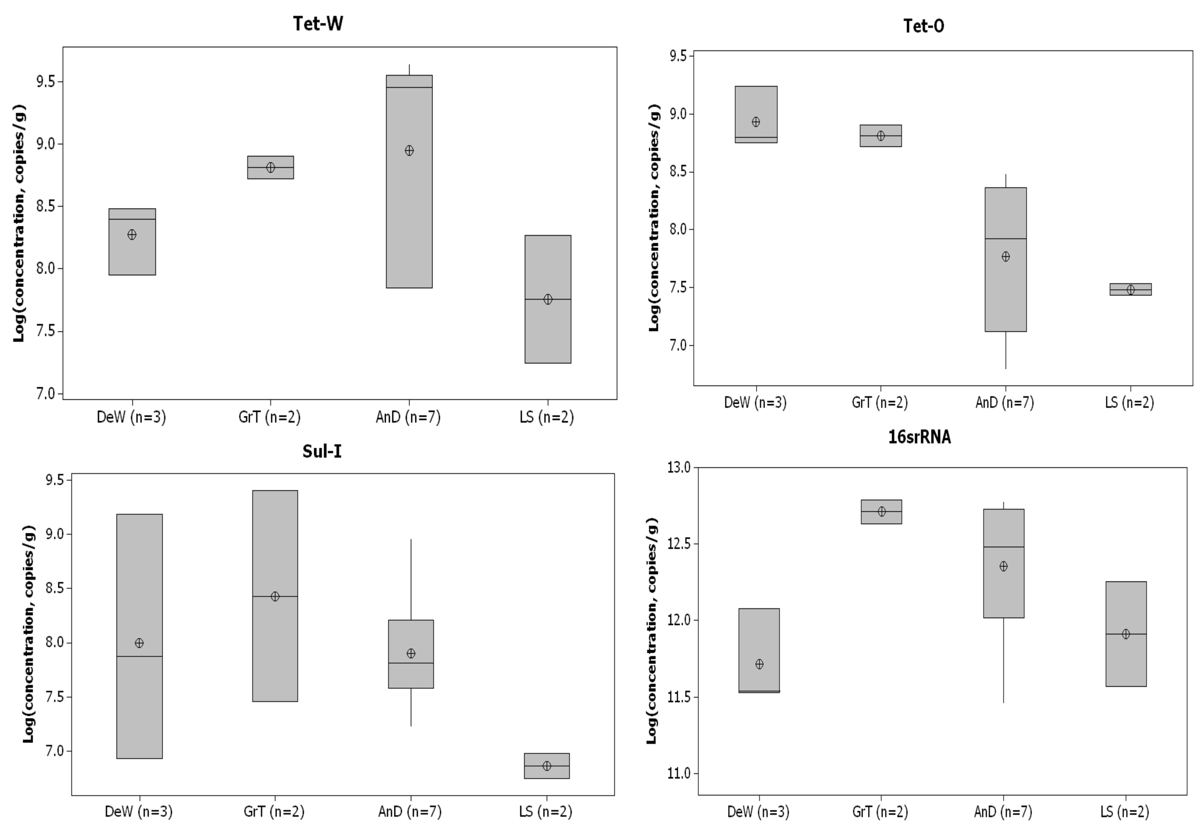

Fig. 3 : Log concentration of tetracycline resistant gene (Tet-W, Tet-O), sulfonamide resistant gene (Sul-I) and 16srRNA gene abundance in biosolid sample of different wastewater utilities. Sludge treatment processes include: DeW=Dewatering; GrT=Gravity Thickening; AnD=Anaerobic Digestion; LS=Lime Stabilization. (n=no. of sampling events)

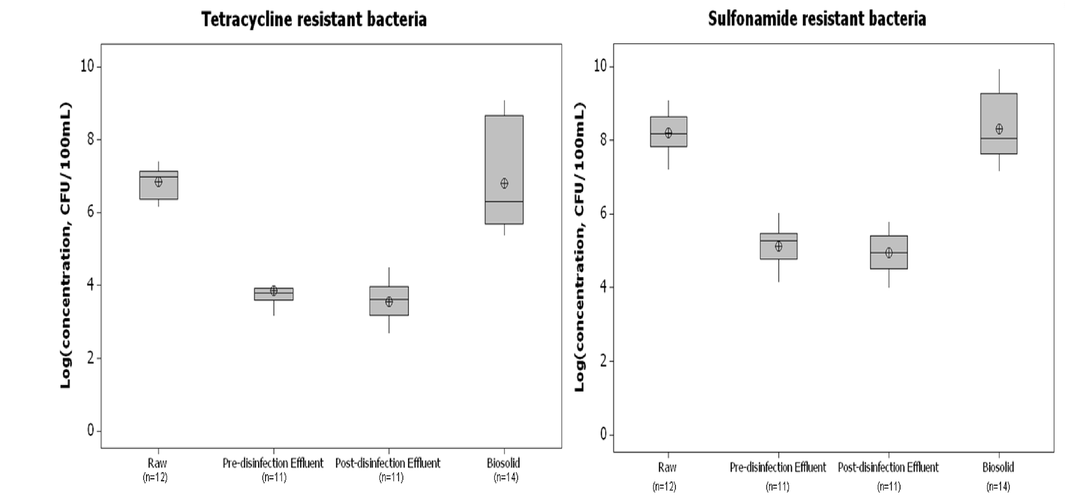

Fig.4 Log concentration (CFUs/100 mL) of tetracycline resistant and sulfonamide resistant bacteria at different sampling points of all the five wastewater utilities.

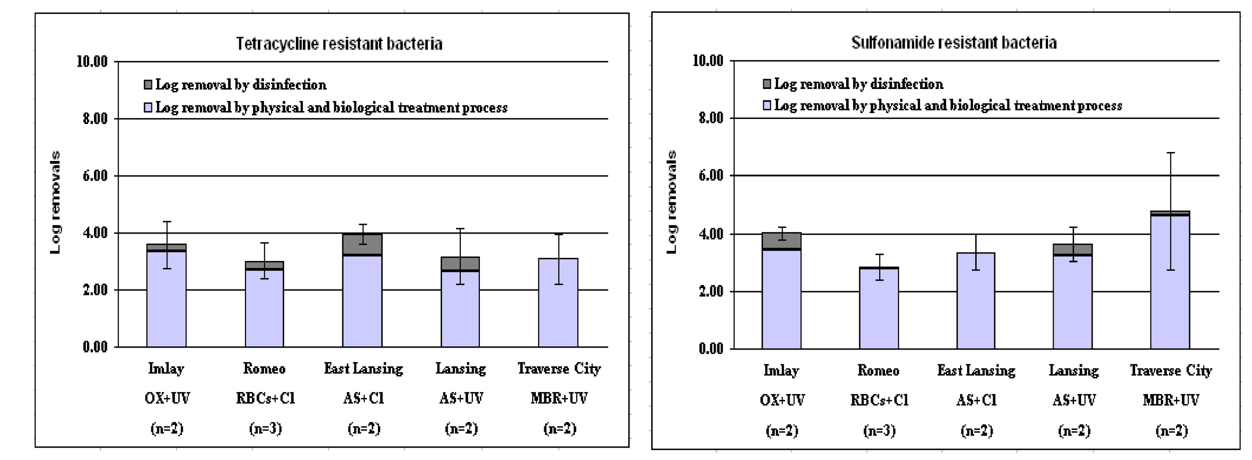

Fig. 5 Log removals of tetracycline resistant and sulfonamide resistant bacteria from wastewater sample of different wastewater utilities. Abbreviations: OX = Oxidative ditch; RBCs = Rotatory Biological Contactors; AS = Activated Sludge process; MBR = Membrane Biological Reactors; Cl = Chlorination disinfection; UV = Ultraviolet radiation disinfection;

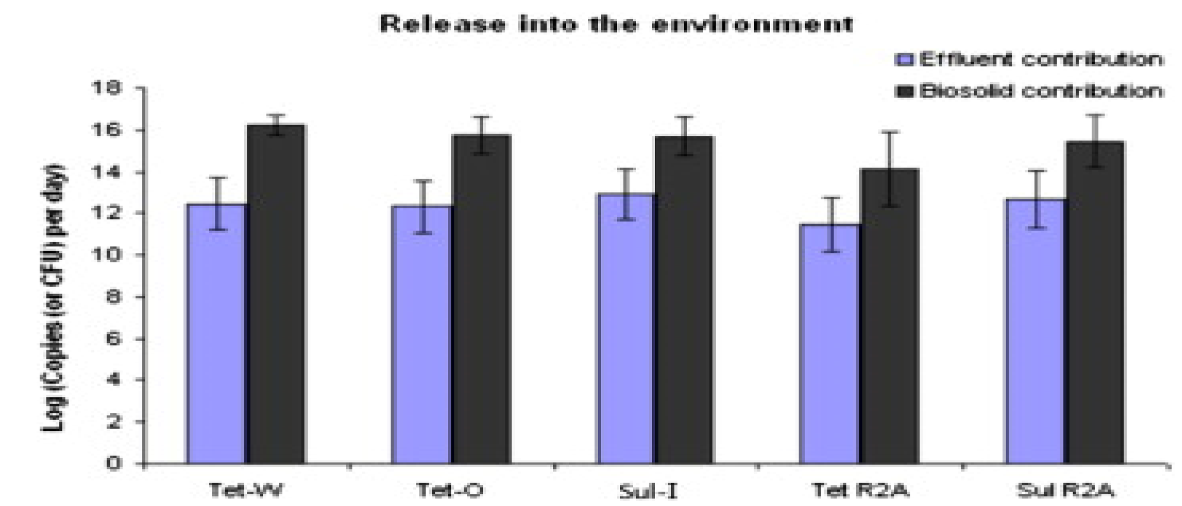

Fig. 6 Release of copies or CFU of ARGs (*Tet-W, Tet-O, SulI*) and ARB (*Tet R2A* and *Sul R2A*) respectively through Effluent and Biosolids into the environment on a daily basis from the WWTPs.

CONCLUSIONS

- Wastewater utilities seem to be a potential sources of emerging tetracycline and sulfonamide resistant genes and -bacteria in our environment.
- Overall, the trends observed in concentration ranges at different sampling points from all the wastewater treatment plants are:

  raw influent > pre-disinfected effluent > post-disinfected effluent
- Disinfection did not prove to have significant contribution to ARGs and ARB reduction
- Concentration of ARGs (tetW and tetO) and 16s rRNA gene in the effluent of an MBR (with a UV disinfection process) utility were **1-3 log** less compared to conventional treatment utilities
- Significant difference ($p<0.05$) was observed in concentrations of both *tetO* and *sul1* genes in biosolids samples between the advanced treatment methods (anaerobic digestion and lime stabilization) and the conventional treatment methods (dewatering and gravity thickening)
- Daily release loads of ARGs and ARB in the environment were found to be higher through biosolids relative to effluents.