Influence of sampling methods on the measurements of urban stormwater quality constituents - Preliminary results

Influence des méthodes d'échantillonnage pour la mesure de la qualité des eaux pluviales urbaines - Résultats préliminaires

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RÉSUMÉ

Le choix entre échantillonnage automatique et échantillonnage manuel de la qualité des eaux pluviales urbaines a été abordé par le passé en termes d'efficacité économique, de sécurité sur le terrain et de sens pratique. Cependant, les expériences montrent que les deux modes d'échantillonnage peuvent produire des données de qualité des eaux pluviales statistiquement différentes. Alors que, par le passé, l'attention portait sur les différences entre les solides échantillonnés, un nouveau problème est abordé dans cette étude, à savoir les impacts potentiels des méthodes d'échantillonnage sur les concentrations en bactéries indicatrices de contamination. A cette fin, quatre bactéries indicatrices de contamination (coliformes, *E. coli*, entérocoques et *C. perfringens*) ont été échantillonnés dans les égouts pluviaux de deux bassins versants urbains à Östersund, en Suède, en utilisant à la fois des échantillonneurs automatiques et l'échantillonnage manuel. Ces données ont ensuite été complétées par des mesures des matières en suspension (MES) totales et de la turbidité, en admettant que les bactéries sont majoritairement transportées en étant fixées à des solides. Les résultats préliminaires indiquent qu'il peut y avoir de grandes différences entre les bactéries indicatrices de contamination des échantillons automatiques et manuels, les mesures d'*E. coli* ayant les écarts les moins importants et les valeurs de la turbidité étant bien corrélées avec toutes les bactéries indicatrices de contamination des échantillons automatiques et manuels, et surtout les mesures d'*E.coli.* Ces résultats serviront à poursuivre cette étude pour améliorer le plan expérimental existant et développer des directives pratiques pour les études de la pollution fécale dans les égouts pluviaux municipaux.

ABSTRACT

The choice between automatic and manual sampling of urban stormwater quality has been addressed in the past as an issue of economic efficiency, field safety, and practicality. Nevertheless, there is experimental evidence that both types of sampling may yield statistically different stormwater quality data. While the past attention focused on differences in sampled solids, a new issue was addressed in this study, the potential impacts of sampling methods on concentrations of indicator bacteria. Towards this end, four indicator bacteria (coliforms, *E. coli*, enterococci and *C. perfringens*) were sampled in storm sewers of two urban catchments in Östersund, Sweden, using both automatic samplers and manual sampling. Such data were further supplemented by measurements of total suspended solids (TSS) and turbidity, recognizing that bacteria are mostly transported in the attachment to solids. Preliminary results indicate that there may be large differences between indicator bacteria in automatic and manual samples, with *E. coli* measurements yielding the least differences, and turbidity readings were correlated well with all the indicator bacteria and particularly *E. coli*. These findings will be used in the continuation of this study for refining the existing experimental design and developing practical guidance for surveys of municipal storm sewers for faecal pollution.

KEYWORDS

Automatic sampling, Indicator bacteria, Manual sampling, Stormwater quality, TSS, Turbidity

1 INTRODUCTION

Stormwater conveys a variety of chemicals and materials which may cause significant impacts on water quality in the receiving waters. Thus, there is a continuing interest in stormwater quality and the methods for its assessment by field sampling and laboratory analysis, or direct on-line measurements in the field by water quality sensors. The former method offers much greater flexibility in terms of constituents studied, but suffers from limitations imposed by sampling bias, discrete data collected sometimes at relatively long intervals, and delays in obtaining the actual data caused while waiting for laboratory analyses. The on-line measurements produce data sampled at short intervals (i.e., measured in seconds), but at the current level of development, the list of constituents that can be measured this way is limited to about half a dozen (including turbidity, dissolved oxygen, pH, temperature, and conductivity) and in the case of UV/VIS spectrometers, additional constituents can be measured as "equivalents" of such conventional parameters as COD (chemical oxygen demand), TSS (total suspended solids), and nitrate (Gruber et al., 2005). When weighing the pros and cons of these two approaches, it would appear that fundamental research of basic constituents measurable on-line benefits from high frequency measurements, but in the studies concerned with mass balances and less common constituents the preference is given to sampling and subsequent laboratory analysis of the collected samples.

Stormwater sampling can be done by means of automatic samplers, or be performed manually. The former method requires a greater initial investment in equipment purchase, but offers lower labour (operational) costs and collection of samples without such limitations as those imposed on manual sampling by the need to dispatch the staff to the sampling sites in inclement weather and regardless of the time of the day. Thus, the choice between the automatic and manual sampling has been mostly addressed as an issue of economic efficiency, field safety, and practicality.

With the exception of the last decade, not much attention has been paid to stormwater sampling techniques and their effects on data uncertainty during the last 30 years. The recent surge of interest was brought about by concerns about the lack of guidance for designing stormwater sampling programs (Harmel et al, 2003), and concerns about stormwater solids monitoring bias caused by the choice of test methods (Gray et al., 2000; Siu et al., 2008; Nordqvist et al., 2011) or the use of automated sampling (Guo, 2007; Gulliver, 2010; Roseen et al., 2011). In the case of solids, the main issue is "under-sampling" of heavier particles which may not be captured by withdrawals of sample aliquots for conventional analyses (e.g., TSS).

However, there is another group of stormwater quality constituents, indicator bacteria, for which the issue of automatic vs. manual sampling is also of concern for different reasons than in the case of solids – the risk of sample cross-contamination in the sampler. Even though the automatic samplers use purge cycles to drain water from the previous sampling operation from the sampling line, some bacteria are likely to stay in the line and this may affect the quality of the next sample. Recognizing the lack of data in this field, a comparative study of automatic and manual sampling of indicator bacteria in stormwater was conducted, and supplemented by sampling of TSS and turbidity, because most bacteria in the water column are attached to solids and the sample transport (particularly where long sampling line overcoming high lift is used) may lead to uncertainties in the transport of solids and thereby of attached bacteria as well. Thus, the main study objective was to determine whether these two sampling methods, automatic and manual, produce statistically significant differences in the measurements of bacteria, TSS and turbidity. In this paper, we present preliminary results of this study including three storm events at which samples were collected.

2 METHODS

2.1 Study area description

The data presented in this paper was collected at storm sewer manholes located close to the sewer outlets in two urban catchments in Östersund, Sweden. The City of Östersund has about 58,000 inhabitants and is located in the central part of Sweden at latitude 63° 11' N and longitude 14° 30' E, with terrain elevations between 300-380 m above sea level. The selected study catchments, Tjalmargatan, further referred as A, and Beijers, further referred as B, are serviced by separate storm sewers with outlets draining into Lake Storsjön, the fifth largest lake in Sweden (area: 464 km^2). The lake is a source of drinking water for abound 50,000 of city inhabitants (16-17 million l/d) and also

serves for recreational purposes. Catchment A is a 20 ha residential catchment with imperviousness of about 50% and is situated 500 m south of the drinking water plant. Even though the storm sewer draining the catchment conveys significant baseflow during the entire year, baseflow samples collected during dry periods have shown no elevated levels of TSS or indicator bacteria. Catchment B is a 40 ha downtown catchment with imperviousness of about 60%. It houses business and commerce buildings, residential and university campus areas, and it is situated 1500 m north of the drinking water plant. Although the storm sewer draining the catchment conveys no baseflow during dry periods, there could be some cross-connections with sanitary sewers upstream of the sampling point, which are activated during rain events.

2.2 Sampling procedure

Approximately 1 L discrete water samples were collected both manually and with automatic samplers at the same sampling point during three moderate storm events, which occurred in September and October, 2012. Rainfall and temperature data was collected by a tipping bucket and a temperature logger, respectively, installed in the city center. Storm events were sampled whenever 2 mm or more of rainfall occurred after at least three antecedent dry days.

Table 1. Climate data for Östersund city centre at the three storm events at which samples were collected.

Storm	Ave. Temp	A. dry days	Tot. Rain	R. Intensity	R. Duration
14.09	9.5°C	14	3.2 mm	3.2 mm/h	1 h
26.09	7.5°C	9	2.8 mm	0.4 mm/h	7 h
04.10	10.5°C	6	6 mm	4.6 mm/h	1.5 h

Both sampling sites were equipped with area-velocity flow meters ISCO 2150 and portable automatic samplers ISCO 6712 to allow flow-weighted sampling. Each sampler was installed on the ground surface next to the sampling manhole. The sampler intake tubing was attached to a stainless strainer positioned 2 cm above the pipe invert to avoid sediment build-up and tube clogging. The length of the sampling tubing from the pipe invert to the sample bottles was 5.5 m and the vertical lift was 4 m at catchment A, and a 3.75 m length with a 3 m vertical lift at catchment B. The typical sampling line flow velocities for vertical lifts of 3 and 4 m were between 0.83-0.87 m/s with pump flow rates around 0.06 l/s. The automatic samplers were manually activated to collect sequential water samples during storm events, every time a predetermined volume of runoff passed through the sampling station. Manual samples were collected from the same sampling location by dipping pre-cleaned bottles, attached to a pole, into the flow, at the same times as the automatic samples were withdrawn. Withdrawals of automatic samples took about 10-15 s, and the manual sampling bottles were immersed in flow for about the same time. Between the storm events, manual and automatic sample bottles were rinsed and autoclaved at 121°C for 15 min, the sampler intake tubing and strainer were washed first with a hot detergent solution, then with 70% Ethanol and rinsed with distilled water. Furthermore manual sampling bottles were rinsed after each sample with distilled water.

2.3 Selected parameters and analysis methods

The commonly used indicator bacteria groups, total coliforms, *E. coli*, int. enterococci, *C. perfringens* and total suspended solids (TSS) have been selected for simultaneous sampling and comparative study purposes. Bacteria samples were preserved in cooling boxes at less than 5°C, and both bacteria and TSS sample analyses were started within 12 h of sample collection. All bacteria samples were analyzed at a local accredited laboratory using membrane-filtration, according to the international standard methods (ISO 8199:2005). Bacteria colonies were counted after 48 h incubation time at 35°C for total coliforms, and 44°C for both *E. coli* and int. enterococci (SIS 28167:1996; ISO 9308-1:2000b; ISO:7899-2:2000c). *C. perfringens* colony units were counted after 24 h incubation time at 44°C under anaerobic conditions (ISO 6461-2:1986). The detection interval for indicator bacteria was 10-300,000 CFU/100 mL with 35% uncertainty for total coliforms and *E. coli*, and 30% and 50% uncertainty for int. enterococci and *C. perfringens*, respectively. TSS were analyzed by standard methods at a local laboratory, filtered through a previously weighed glass fibre filter (Whatman GF/A filter), dried at 105°C for at least 1 h and weighed again (SS-EN 872:2005), with the lower detection limit of 5 mg/l and 15% uncertainty. Turbidity was measured with a Hach portable Turbidimeter 2100.

3 RESULTS AND DISCUSSION

A summary of sample collection dates and the constituents analyzed, in catchments A and B, appears in Table 2. The entire data set includes 60 and 40 samples collected in catchments A and B, respectively, which were analyzed for indicator bacteria. TSS and turbidity were analyzed and compared in about 50% of the samples in catchment A and 80% of the samples in catchment B.

Table 2. Total number of samples (manual and automatic) collected during individual storm events in catchments A and B.

Storm	Bacteria		TSS		Turbidity	
	A	B	A	B	A	B
14.09.2012	18	18		10		12
26.09.2012	22	14	22	14	18	14
04.10.2012	20	8	10	8	10	8

All sampled data are presented graphically in Figs.1-3, in plots of automatic sample data vs. manual sample data, combined for both catchments, A and B. In such graphs, the 45º line represents perfect fit; all data above the line indicate higher concentrations in automatic samples compared to the manual ones, and vice versa. Furthermore, deviation lines ± 25% and ±50% are shown as well. The data are presented in the following order: Fig. 1 displays total coliform and *E. coli*, Fig. 2 displays int. enterococci and *C. perfringens*, and, finally, Fig. 3 displays TSS and turbidity data.

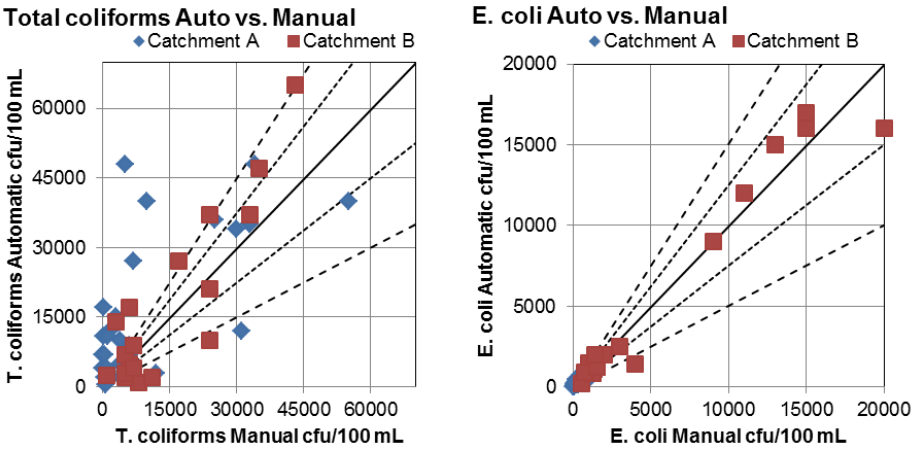

Figure 1. Left panel: total coliform counts (CFU/100 mL) in automatic samples vs. coliform counts (CFU/100 mL) in manual samples; Right panel: *E. coli*. counts (CFU/100 mL) in automatic samples vs. *E. coli* counts (CFU/100 mL) in manual samples. Dashed lines indicate 25 and 50% deviations from perfect fit.

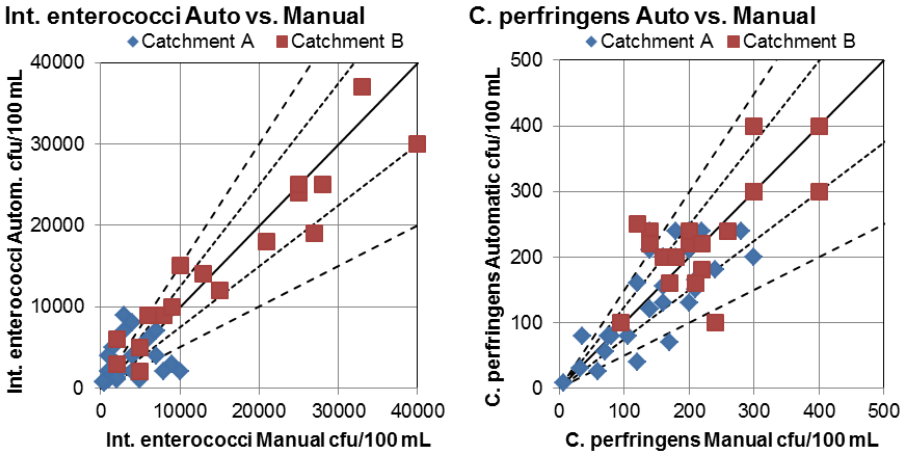

Figure 2. Left panel: int. enterococci counts (CFU/100 mL) in automatic samples vs. int. enterococci counts (CFU/100 mL) in manual samples; Right panel: *C. perfringens* counts (CFU/100 mL) in automatic samples vs. *C. perfringens* counts (CFU/100 mL) in manual samples. Dashed lines indicate 25 and 50% deviations from perfect fit.

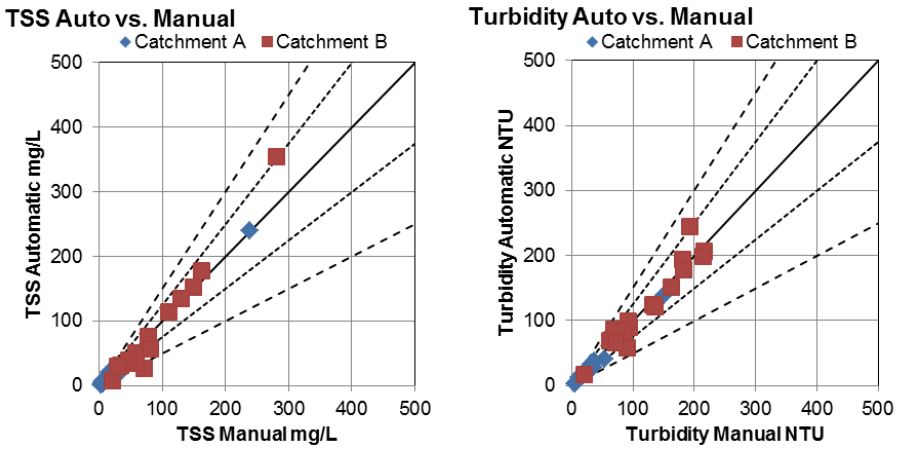

Figure 3. Left panel: TSS concentrations (mg/L) in automatic samples vs. TSS concentrations (mg/L) in manual samples; Right panel: Turbidity readings (in NTU) in automatic samples vs. turbidity readings (in NTU) in manual samples. Dashed lines indicate 25 and 50% deviations from perfect fit.

Discussion of data follows the order of presentations in Fig. 1-3. In the overall assessment, there are differences between the data from automatic and manual samples, but their magnitude greatly varies, depending on the constituent addressed. In the case of coliforms, the differences between counts in automatic and manual samples are relatively large, particularly in Catchment A, and frequently exceed the bands ±50%. In general, the automatic samples appear to produce higher coliform counts, which may be explained by the ubiquity of coliforms and the notion of coliform contamination of the sampling line and other internal sampler surfaces when more contaminated water is passing through. Such high readings then elevate the subsequent sample readings. Uncertainties in faecal indicator bacteria counts related to sampling line contamination are to be further addressed in an ongoing study by the authors. Generally, coliforms are very common and do not represent a strong indicator of faecal contamination.

E. coli produced a different response, which can be characterized by relatively close agreement between the counts in automatic and manual samples, and hardly any bias in both types of samples, which would tend to agree with the findings of McCarthy et al. (2008). The magnitude of counts is also of interest, with relatively low readings in catchment A (the smaller residential catchment with some clean baseflow) and fairly high readings in catchment B, which is a larger downtown catchment without any baseflow. In the latter case, the peak value of *E. coli* reached levels of 1.7-2 x 10^4 CFU/100 mL, which are indicative of the presence of other than stormwater sources. There may be cross-connections in the system, and such an eventuality will be further addressed in future sampling at this site.

Int. enterococci (Fig. 2) produced relatively good agreement between automatic and manual samples, with counts in catchment B exceeding those in A by a fair margin (factor of 4), again possibly pointing to external sources of these indicator bacteria at this site.

C. perfringens produced much higher scatter, with some points falling outside of the ±50% bands. In this case, catchment B again produced higher counts, but by a much smaller margin.

In general, some advantages of using a battery of indicator bacteria are emerging, within the uncertainties inherent to this relatively small data set. In particular, some indicators are less susceptible to cross-contamination in automatic sampling; in this study, it appears to be *E. coli* and int. enterococci, which showed a much better agreement between both types of samples, than coliform and *C. perfringens*. Such a response may arise from specific properties of the indicator bacteria studied and will be further addressed in the continuation of this study.

TSS and turbidity are plotted in Fig. 3 and produced comparable results, when comparing automatic and manual samples. The observed TSS values were relatively low, with a maximum concentration being less than 300 mg/L and slightly higher concentrations in manually collected samples in the downtown catchment B. Huang et al. (2010) reported similar variances related to the sampling location (inlet, outlet). A USGS study (1999) showed negligible deviations between two comparable methods though applied in the collection of surface water samples. The lower concentrations in automatic

stormwater samples may be explained by the sampling height and lower capacity to lift heavier particles. However, further uncertainties related to the flow rate and velocities need to be considered. Turbidity readings agreed almost perfectly, which is not surprising, because such readings should not be affected by coarser solids not sampled. In future observations, it is planned to verify the particles size distributions in stormwater samples at these site to confirm that indeed only fine grained solids are sampled. Furthermore, higher TSS readings in catchment B confirm that this catchment should produce higher bacterial counts, recognizing that bacteria are transported mostly in the attachment to solids. Finally, while Figs. 1-3 provided a good comparison of concentrations in automatic vs. manual samples, magnitudes of differences between both types of samples are not that well recognizable in these graphs. Consequently, the data were also re-plotted in another form – as histograms of relative differences between the automatic and manual sample concentrations (i.e., $\Delta = (C_A - C_M)/C_M$), where C is concentration and subscripts A and M refer to automatic and manual samples, respectively.

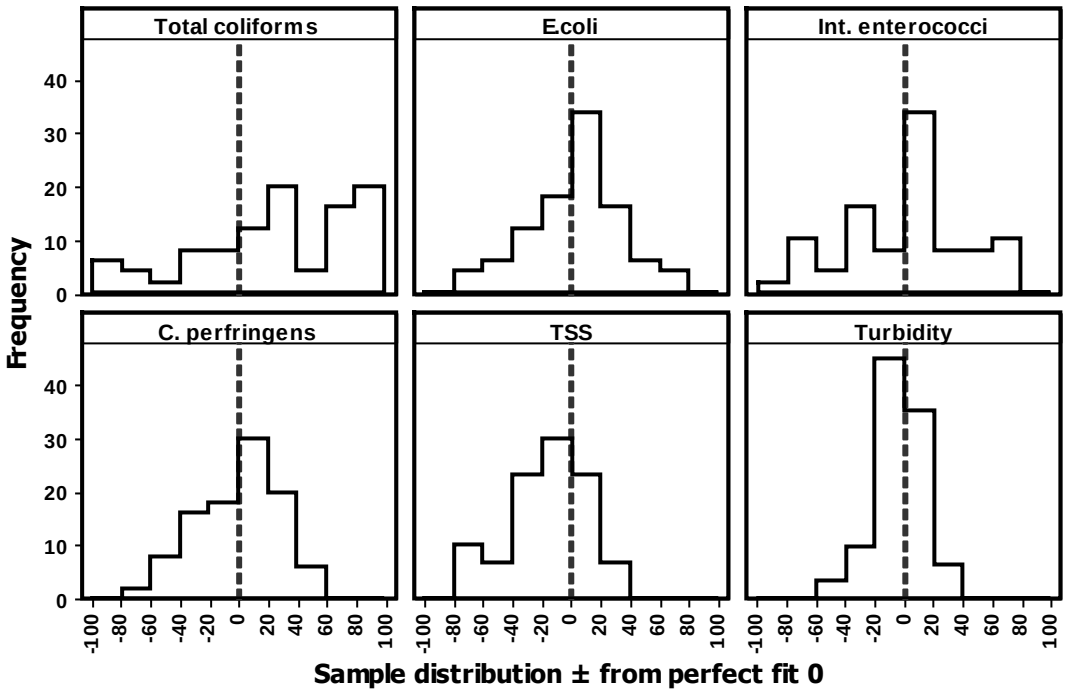

Figure 4**.** Histogram of the differences between automatically and manually collected samples in percent. Positive values indicate higher concentrations in the samples taken with automatic samplers and vice versa.

Fig. 4 shows comparable results to those in Figs. 1-3, but with a greater clarity of indicating which sampling method produced higher concentrations. Three types of responses can be discerned in Fig. 4; concentrations from automatic samples exceeding those in manual samples ($C_A > C_M$), both types of samples producing comparable results ($C_A \sim C_M$), and concentrations in automatic samples under-representing those in manual samples ($C_A < C_M$). Coliform and int. enterococci belong to the first category ($C_A > C_M$), *E coli*, *C. perfringens* and turbidity belong to the second category ($C_A \sim C_M$), and finally TSS belong to the third category ($C_A < C_M$). This would indicate that TSS might be under-sampled by the automatic sampler. These findings are in agreement with those offered and discussed earlier in this section.

A Paired *t*-test was performed to determine if significant differences existed between the sampling methods. The results of such assessments are in Table 3, which shows the mean ± standard deviation for the automatically and manually collected samples and the p-value of paired *t*-test. The paired t-test was conducted both for the whole data set and separately for three sub-sets in which the data was allocated depending on the samples with low, medium and high concentrations. All samples were divided into three categories according to the sample distribution for each water constituent, with approximately the same number of samples classifying 30 to 35% of the samples with the lowest highest and medium concentrations into the low, high and medium category, respectively.

Table 3. Mean concentrations ± standard concentrations of all samples and the three sub-sets (low, medium and high) collected in catchment A and B and p-values of the paired t-test. p <0.05** and p <0.10*.

Parameter	Auto ± SD	Manual ± SD	P-value paired *t*-test	Sample No. Manual + Auto
T. coliforms All	19200± 20042	15672±21700	**0.046****	100
T. coliforms Low	2813±1413	4757±3773	**0.078***	32
T. coliforms Medium	10765±3364	6148±8479	**0.054***	34
T. coliforms High	43059±16369	35471±26924	**0.093***	34
E. coli All	3234±3416	6882±7155	0.425	104
E. coli Low	162±85	179±133	0.556	32
E. coli Medium	568±215	550±282	0.702	38
E. coli High	9313±9836	9856±10099	0.437	34
Int. enterococci All	10967±11302	18848±17189	0.703	100
Int. enterococci Low	1545±532	2558±2556	**0.083***	38
Int. enterococci Medium	4786±1805	4179±2431	0.468	28
Int. enterococci High	26588±26208	36941±22309	0.887	34
C. perfringens All	180±181	89±86	0.920	100
C. perfringens Low	78±38	108±64	**0.021****	32
C. perfringens Medium	183±22	189±44	0.649	32
C. perfringens High	268±56	239±84	**0.046****	36
TSS All	56 ±58	79 ±68	0.365	64
TSS Low	7.5±3.9	11±6.4	**0.028****	24
TSS Medium	30±6.2	40±15	0.031**	22
TSS High	151±98	143±75	0.447	18
Turbidity All	82±84	69 ±67	0.499	62
Turbidity Low	18±8.0	20±9.4	**0.021****	22
Turbidity Medium	66±24	69±23	0.571	20
Turbidity High	168±42	168±35	0.965	20

When comparing whole combined sets of data (i.e., non-stratified sets), the *t*-test confirmed a significant (95% CI) difference only for total coliform concentrations with 15,672 and 19,200 mean coliform concentrations in manual and automatic samples, respectively. For all other parameters and whole sets of concentrations, no significant differences were noted. On the other hand, the sub-sets with low concentrations showed significant differences in all cases, total coliforms (90% CI), int. enterococci (90% CI) and *C. perfringens* (95% CI), except for *E. coli*.

Finally, a correlation matrix was calculated for manually collected samples and is presented in Table 4 below.

Table 4. Pearson correlation coefficient *r* and *p-value* for parameter concentrations in manually collected samples. P<0.05: bolded font.

Parameter	T. coliforms	E. coli	Int. enterococci	C. perfringens	TSS
E. coli	**0.797** ***0.000***				
Int. enterococci	**0.505** ***0.004***	**0.621** ***0.000***			
C. perfringens	0.157 *0.407*	0.107 *0.575*	0.313 *0.092*		
TSS	0.305 *0.101*	**0.481** ***0.007***	0.328 *0.076*	0.021 *0.913*	
Turbidity	**0.453** ***0.012***	**0.710** ***0.000***	**0.544** ***0.002***	0.285 *0.127*	**0.859** ***0.000***

The data in Table 4 indicates some useful trends which will be further utilized in continuation of this study. The highest correlation (r =0.859) was found between TSS and turbidity, which has been frequently reported in the literature (e.g., Gruber et al., 2005) and may be used in replacing TSS tests by on-line turbidity readings. Furthermore, turbidity yielded higher *r* values for all the four indicator bacteria than TSS, with the highest value of r =0.710 for *E. coli*. Pending further verifications, this will guide future work on indicator bacteria detections by using turbidity readings as a surrogate parameter, pending further verifications against more extensive field data. Finally, a high value of *r* for *E. coli* and coliform was also noted but it does not have practical value – both indicators belong to the same family of bacteria and thus their correlation is to be expected. In the overall evaluation, the results obtained for two types of samples, automatic and manual, indicate significant differences for some ranges of the most parameters studied. The concentrations of various parameters may be biased by uncertainties caused by several factors, including sample cross-contamination in the sampler, bacteria build-up and die-off in the sampling equipment, sampler cleaning, sampling lift and sample storage. Harmel et al. (2010) showed similar findings with varying concentrations for samples withdrawn from small streams by two similar sampling methods. The higher coliform concentrations in automatic samples suggest higher uncertainties for these particular indicator bacteria when studied in stormwater. Regardless of the cleaning procedures between sampling events, baseflow occurring during dry weather may contribute to a build-up of coliform bacteria from natural sources between sampling events close to the sampling intake. With this assumption, the automatic purging of the sampling line prior to sample withdrawal may not be sufficient in the case of ubiquitous indicator bacteria, like total coliforms. The three other indicator microorganisms show lower deviations, with the same trends, between manual and automatic samples, suggesting that similar issues need to be addressed for automatic sampling methods. Low variation was indicated for *E. coli* suggesting that both sampling methods can be considered reliable, when working with this indicator. Uncertainties related to automatic samplers reported by McCarthy et al. (2008) showed negligible differences in indicator bacteria with respect to the sampling point location (on the bottom or water surface), however other factors have not been investigated.

4 CONCLUSIONS

A comparative study of automatically and manually collected samples in the same cross-section of storm sewers in two test catchments, at the same times, produced the findings indicating statistically significant differences between both types of samples, for a list of six stormwater quality constituents: four types of indicator bacteria (total coliforms, *E. coli*, int. enterococci, and *C. perfringens*), and TSS and turbidity. Among the indicator bacteria, different responses were found; with coliforms being positively biased in automatic samples, but the remaining three indicators producing comparable results, and *E. coli* and int. enterococci indicating relatively small differences between the both types of samples. These finding are helpful for selecting the best indicator in the ongoing studies in the Swedish city of Östersund. Different responses were observed for TSS and turbidity, with automatic samples “under-sampling” TSS (probably missing some coarser solids) and turbidity producing highly similar readings. Explanations of such results vary from parameter to parameter; for indicator bacteria, biases may be introduced by uncertainties in the sampling methods and the risk of sample cross-contamination in automatic samples. Other factors may include bacteria build-up and die-off, transport of solids with attached bacteria, equipment cleaning and sample storage. Under-sampling of TSS appears to be related to the pick-up of solids from the sewer flow and a reduced capacity to transport coarser solids in the sampling line to higher elevations. However, further investigations of the sources of errors and uncertainties will require working with a much more robust dataset with many more storm events and samples, than currently available.

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