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FINAL REPORT SWITCH-LODZ / RTD 2007 M: 13-24	
WG VII: Ecohydrology and Urban Aquatic Ecosystems	
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Content:	Identification of anthropogenic contaminations in the Sokolowka river catchment: - identification of the main threats (red points) of Escherichia Coli and nitrates along the river system (at 14 stations).

Summary

The aim of research was to identify the main threats (red points) of *Escherichia Coli* and nitrates along the Sokolowka river ecosystems. The investigation was carried out at the fourteen sampling stations are located on the river and five on reservoirs.

In the upper course next to Folwarczna Street, where the first research station is, we can discover avery high MPL (Most Probable Number) of E. coli, more than > 1600 MPL/100ml of water, which classifies this part of the river as belonging a third class of river cleanliness. This high contamination in initial stations can result from the pollution by illegal inflow from leaky cesspools that aresituated near detached houses. Along the river course we can see a gradual reduction of E. coli. In the middle part (st. 6, 7, 8) we can classify the river as belonging a first class of river cleanliness. Following the lower course of the river contamination increases, resulting in a decline in classification of river quality class (being again classified to a third class). In last stations we can again see a contamination which can result mainly form leaky cesspools. Increasing degradation can also be a result of agricultural using of the catchment in the lower part of the river. That is why contamination by E. coli can stem from leaky cesspools, storage manure or application of liquid manure in the field. Because of this, especially in the area of stations 1-4 and 10-14, a combined flow constructed wetland system should be implemented. (Bis et. al. 1997; Helman-Grubba & Marcinkowski, 2006).

Background

The studies were carried out at the fourteen stations located on the river and five on reservoirs. Possible pollution way on this selected representative stations are described below:

St.1. The place is near to the detached house, which can contaminate the river constantly by means of leaky cesspools or an illegal inflow containing detergents (soapsuds floating on the water can often be seen in this area).

St. 2. Close to the first spot there is a park where people walk their dogs.

St. 3, 4, 5. In the park, beside a number of people walking their dogs, there are ponds where some people go fishing.

St. 6, 7. The above places are situated on the area \belonging to ahousing estate. In this area there is a park for dogs which may possibly be the reason of a high concentration of *E. coli*.

St.8, 9. (in the park also but about 500-700m next to the housing estate) These places are also in the park so the contamination can have the same source. Additionally, there is a factory nearby that contaminates the river mostly by chemicals (soapsuds floating on the water can be seen) and possibly by a certain amount of biowastes (from the toilet).St.10, 11. Next to the houses with gardens and small agricultural activity. The contamination may originate leaky cesspools or application of liquid fertilisers.

St.12. The area described is a typical agriculture area, featuring lots of houses, fields, gardens.

St.13, 14. This area is also used as an agricultural terrain and a site for riding horses, so there are many stables there.

Materials and methods

The samples were collected usually 3-4 times per season. In the collected samples of water Fecal Coliforms and *E. coli* summary were detected using Colitag ™ tests.

Colitag™ MPN Instructions

Product Overview

Colitag™ is a selective and differential medium for the enumeration of total coliforms and *E. coli* in drinking water, surface, and source water samples. It is a one-step, ready to use medium, to be combined with a water sample. This product is designed to test water or other samples for coliform and/or *E. coli* bacteria in 24 ± 2 hours and does not require further confirmation or verification steps. Colitag™ detects 1 colony-forming unit (CFU) of *E. coli* and other coliform bacteria. The density of coliforms and *E. coli* is estimated by use of the Most Probable Number (MPN) method as described in the *Standard Methods for the Examination of Water and Wastewater, Section 9221, 20th Ed.*

Method Summary

The ColitagTM method is based on the detection of two enzymes, ß-glucuronidase and ß-galactosidase, which are characteristic of *E. coli* and the coliform groups, respectively. ColitagTM detects total coliforms using the chromogenic substrate ortho-nitrophenyl-ß-D-galactopyranoside (ONPG). Upon hydrolysis by ßgalactosidase, ONPG produces a distinct yellow color, confirming the presence of coliforms in the sample. For detection of *E. coli*, Colitag™ utilizes the fluorogenic enzyme substrate 4-methylumbelliferyl-ß-Dglucuronide (MUG). Upon hydrolysis by ß-glucuronidase, MUG releases 4-methylumbelliferone. This reaction by-product fluoresces when exposed to UV light. The ß-glucuronidase enzyme is specific to *E. coli* and observation of fluorescence differentiates this organism from other members of the coliform group.

MPN Test Procedure for Total Coliforms and MUG-Positive *E. coli* Bacteria

Method A:

1. Select the appropriate number of tubes per sample for the MPN test (10 tubes x 10 ml or 5 tubes x 20 ml).
2. Aseptically add 100 ml of a water sample to the Colitag™ medium.
3. Divide the sample into the appropriate number of tubes.
4. Incubate tubes at 35.0 ºC ± 0.5 ºC for 24 ± 2 hours.
5. MPN values are available in Tables 9221:II. and 9221:III. in the 20th Edition of the *Standard Methods for the Examination of Water and Wastewater*, page 9221-5.

Method B:

1. Dissolve Colitag in sterile distilled water to obtain a desired quantity of the medium.
2. Perform serial dilutions and inoculations according to the MPN method described in Section 9221 of the *Standard Methods for the Examination of Water and Wastewater, 20th Ed.*
3. MPN values are available in Table 9221:IV of the same reference (see footnote 1).

1 Method described in the 20th Edition of the *Standard Methods for the Examination of Water and Wastewater*, Section 9221 C, 20th Ed. *Sample Interpretation*

1. Visually check each sample/tube for yellow colour. If the test sample is yellow, then coliform bacteria are present.
2. Examine the solution for fluorescence using a long wavelength (366 nm) UV lamp. If a bright blue fluorescence is observed, *E. coli* bacteria are present.
3. To enumerate total coliforms and *E. coli* present, use the combination of positive and negative tubes to select the corresponding value from the appropriate MPN Table 9221:II, 9221:III or 9221:IV (*Standard Methods for the Examination of Water and Wastewater*, 20th Ed.). Tables contain the MPN Indices and 95% Confidence Limits for All Combinations of Positive and Negative Results.

Results

Stations	**Index NPL/100ml**					
	12.02.2007	**25.08.2007**	**21.09.2007**	**13.11.2007**	**12.12.2007**	
1	24 000	> 1 600	> 1 600	> 1 600	> 1 600	
2		> 1 600	130	> 1 600	920	
3		> 1 600	130	11	> 1 600	
4	130	> 1 600	130	4,5	79	
5		920	79	7,8	6,8	
6		33	33	2	34	
7	230	49	2	2	240	
8		110	33	49	1600	
9		> 1 600	920	22	920	
10	70 000	1600	920	540	> 1 600	
11	2 300	920	> 1 600	1600	> 1 600	
12		> 1 600	160	350	> 1 600	
13	6 200	1600	920	79	920	
14		540	920	17	920	

Discusion

According to the disposal of Minister of Environment from 2004 for “Water quality indicators in river cleanliness class”, Sokolowka River in all its course is classified as belonging to different river quality classes. In the upper course next to Folwarczna Street, where the first research station is, we can see a very high MPL (Most Probable Number) of E. coli, more than > 1600 MPL/100ml of water, which classifies this part of the river to a third class of river cleanliness. For the third class of river MPL should be between 500 to 5000. The high contamination in initial stations can result from the pollution by illegal inflow from leaky cesspools, situatednear detached houses. Along the river course we can see a gradual reduction of E. coli. In the middle part, at stations no. 6, 7 and 8 we can classify the river to the first class of river cleanliness. Microbiological indicator for the first class is 50 MPL/100ml of water. Following the lower course of the river an increase of contamination can be observed, resulting in the decline in classification of river quality class. In this part the river is again classified as belonging to a third class. In last stations we can again see a contamination which can result mainly form leaky cesspools. Increasing degradation can also be a result of \agricultural using of the catchment in the lower part of the river. That is why contaminations by E. coli can stem from leaky cesspools, storage manure or application liquid manure in the field. Because of this, especially in the area of stations 1-4 and 10-14, a combined flow constructed wetland system should be implemented, (Helman-Grubba & Marcinkowski, 2006).

Future measurements may help to show differences in pollutants' content inthe environment during different periods of theyear. We can now see that in the summer period the contamination is lower. It can be because people take their time off, travelling, having holidays. To confirm this opinion, however, further studies should be conducted. .

Depending on the season we can see 2 maxima: spring and summer. In the spring when water temperature starts to increase and the water itself contains lots of nutrients we notice an intense increase in the number of microorganisms, especially microbial. Havingrun out of nutrients, bacteria gradually decline. In the stations with regular inflows of sewages there is not so easy to see this seasonal minimum and maximum number of microbes (Libudzisz et. al. 2007). In some cases a decrease of bacteria content in the river is a consequence of antagonistic reactions with another microbes or toxic substances, like bacteriocins, which are produced by some bacteria, or cyanotoxins, which come from a decay of Cyanophyta (blue – green algae). According to Libudzisz (2007) pH has a significant influence on a decrease of bacteria content. High oscillation of pH has negative relation for E. coli. In our work we can see that notable variation in pH causes a decline of E. coli in water, especially in the summer period.

We should really take into account the risks related to theriver contamination by microbes and lead future measurements, because people use water for many reasons, for example for swimming or drinking without boiling, being unconscious of the risks this may constitute to their health. Future measurements could help to show differences in pollutant content of the environment in different periods of the year.

Conclusions and next steps

The research on the Sokolowka river has enabled to identify main threats (red points) of *Escherichia Coli* and nitrates along the river ecosystems.

This highest contamination of *E. coli,* which classified this part of the river to a third class of river cleanliness, was found in the upper part of the river - because of pollution by illegal inflow from leaky cesspools situatednear detached houses. Along the river course we can see a gradual reduction of *E. coli*. In the middle part (st. 6, 7, 8) we can classify the river as belonging to a first class of river cleanliness. Following the lower course of the river contamination increases, resulting in a decline in classification of river quality class (being again classified to a third class). In last stations we can again see a contamination which can result mainly form leaky cesspools. Increasing degradation can also be a result of agricultural using of the catchment in the lower part of the river, leaky cesspools, storage manure or application of liquid manure in the field. Because of this (next step), especially in the area of stations 1-4 and 10-14, a combined flow constructed wetland system should be implemented. (Helman-Grubba & Marcinkowski, 2006). In order to reduce a high concentration of nitrates in the agricultural part of the catchment we suggest to make a “denitrification wall”/denitrification process and set up phytoremediation work connected with planning wetlands. This could catch nitrates and transform them in a denitrification process into N_2. Reclamation of the river would createnew conditions and improve the environmental quality of existing green areas, the factors linked with the improvement of human health and development in these areas.

Literature:

1. Helman-Grubba M., Marcinkowski M.J., 2006 Technically unconventional possibilities of water protection and remediation of degraded water reservoirs (based on example of technological experience and quotation of ekol-unicon ltd.)
2. Libudzisz Z., Kowal K., Żakowska Z, „Technical microbiology – microorganisms and their habitats” vol.1 Warszawa, 2007, PWN.
3. Minister of Environment enactment from 2004, official no. 32, annex no. 1 “Water quality indicators in river cleanliness class in surface water”
4. Standard Methods for the Examination of Water and Wastewater, Section 9221, 20th Ed.
5. Bis B., Krauze K., Bednarek A., Zalewski M. (1997): Ecological basis of river renaturalization in aspect of reduction the nutrient transport to dam reservoirs and near-shore waters; In : Zalewski M. (ed): Applying of biotechnology for improvement of water quality. Zeszyty Naukowe Komitetu Człowiek i Środowisko; 18: 137-155. Warsaw.