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Research Article

STUDYING MICROBIOLOGY OF RAIN WATER FOR OF THEIR USE IN ECONOMY

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ABSTRACT

Growing areas of sealed surfaces, rising water needs due to industry development, increasing populations, and climatic changes affect precipitation patterns and form a vision of the future in which meteoric water storage may become almost an obligatory activity. The aim of this paper was to identify the amounts and, to some degree, the quality of microorganisms present in rainwater collected from different types of rooftops of utility buildings in the spring-summer season. Apart from the classic culture plate method complemented by flow cytometry. The results of performed analyses explicitly show that rainwater collected from rooftops and directly from air prove to be microbially contaminated to a substantial degree, which includes pathogenic coliforms and faecal streptococci. Waters collected after dry periods also contained bacteria like *Clostridium perfingens*. The findings rule out the possibility of using rainwater collected from roof surfaces of utility buildings before its treatment.

Keywords: rainwater, microbial contamination, flow cytometry, plate method

INTRODUCTION

According to a definition found in Water Resources Law of July 18th 2001 (Journal of Laws no. 115, point 1229 as amended), rainwater is considered in entirety as waste, and whether it is considered waste or not relies on the place of its origin – art. 9, point 14c. When draining rainwater off to sewage we deal with rainwaters coming from a) surfaces permanently sealed, in which case the Water Resources Law classifies such water as waste and b) roofs, in which case the Water Resources Law does not include such water in the waste category. Within the meaning of the law, it can be stated that rainwater from roof is not "waste" regardless of the type of roofing and regardless of the type of building, because the rooftop "surface" is not a "pavement", which is part of the definition of so-called rain "waste" (art. 9, par. 1, point 14, .letter c Water Resources Law; art. 3 point 38, letter c Environment Protection Law). This does not mean, however, that water is supposed to be considered free from chemical or microbial contamination.

Quality tests of rainwater to see if it is fit for use as a source of drinking water and for household/utility purposes indicate the same possibility. Normative values for heavy metals have not been exceeded. The extensive studies into the quality of meteoric water is not in keeping with the broad knowledge on its microbiological aspects despite the fact that the WHO (2004) recommends that water used for household purposes contain no more coliforms than 10 CFU/100 ml in 95% of tested samples. Only few researchers study bacteriology and present adequate findings. They are concerned mostly bacteria which renders water unfit for human consumption. These bacteria are *Escherichia coli* and faecal streptococci [Lee i in., 2010; Amin i Han, 2011; Kaushik i Balasubramanin, 2012]. Very few researchers include in their studies of pathogenic strains such as: *Staphylococcus sp., Pseudomonas aeruginosa, Pseudomonas syringae, Streptococ-*

cus sp., Klebsiella pneumoniae, or *Clostridium perfrigens* [Nawaz i in., 2012, Gikas i in., 2012]. Coliforms and *Escherichia coli* always exceed normative values in the tested samples of rainwater collected from rooftops or terraces. The studies also included tests of rainwater from water collection system against the presence and count of *Campylobacter*- and *Legionella*-type bacteria. The results for most samples showed the amounts of bacteria merely on the level of detection (1 CFU/100 ml of water).

Moreover microbiological research methods for rainwater leave a lot to be desired. Often, the conclusions over the quality of rainwater rely on results obtained with simple methods such as agar plate method or even tube fermentation techniques [Helmreich i Horn, 2008].

The studies employed advanced test methods to study the microbial quality of rainwater collected from different types of rooftops. The findings helped to evaluate the possibilities of utilizing rainwater for household purposes or for agriculture.

RESEARCH METHODS

The studies were carried out in the spring-summer season in 2015. Water was collected from roof gutter with different coats and inclination angle: B – concrete tile (45°), C – ceramics tiles (45°), O – galvanized sheet (45°), and E – epoxide resin (terrace – 2°). For control purposes, airborne water was collected – P. All five points of rainwater collection were at one of Rzeszów's suburban neighbourhoods of detached houses located in the near vicinity of farming land (50 to 200 m distance), and mechanical-biological municipal waste treatment plant (1500 m). Samples were collected from each rainfall that occurred into sterile polyethylene containers opened during rain. Taking into account the washing away effect, portions of water were collected only after at least an hour-long, intensive fall. The samples were transported to the laboratory in a portable fridge in less than 6 hours. For each collected sample of water a series of microbiological assays (Table 1).

Coliforms and *E. coli* were assayed in a standard way using procedures provided for by the PN-EN ISO 9308-1 and PN-EN ISO 9308-2 norm. Likewise, assays were performed for faecal streptococci PN-EN ISO 7899-2. Bacteria from the coli group and *E. coli* were additionally assayed with the use of a chromogenic medium. The medium makes the coli bacteria grow fast due to a combination of proper peptones and the MOPS buffer. The combination of two chromogenic substrates reveals all intestinal bacteria and *E. coli.* Total number of bacteria was determined in accordance with PN-EN ISO 6222 also with an additional variant, where cultures are grown on agar supplemented R with extended incubation. Samples for cytometry analysis were prepared according to the own procedure. The difference between the number of all particles (assayed with Sybr Green) and the number of dead particles (assayed with propidium iodide) provides the overall number of living microorganisms.

RESULTS AND DISCUSSION

The conducted studies indicate the presence of considerable amounts of pathogenic bacteria in rainwater flowing over different rooftops as well as those collected directly from air [Cho and Hwang, 2011; Polymenakou et al., 2012]. In early spring season they are, however, much scarcer compared to warmer seasons. Similar correlations between air temperature and surface from which precipitation is collected has been observed by Lee et al., 2010, Amin and Han, 2011, arrived at similar numbers for coliforms and faecal streptococci in spring and summer seasons (Tables 1, 2, 3).

Also, research on the microbial quality of rainwater from tanks showed that 70% of samples contained excessive amounts of *E. coli* bacteria and faecal enterococci [Ahmed 2014]**.** Some authors also note the very high concentration of these bacteria – up to 500 cfu/100 ml [Wilbers at al., 2013]

In early spring the source of highest contamination levels were concrete tiles and epoxide resin covering a terrace. Water collected from these surfaces after the winter period was found to contain active cells of anaerobic bacteria - *Clostridium perfingens* (Table 2). These organisms are an indication of an old faeces contamination and their presence on roof surfaces is rather rare. They may appear after longer periods with a lack of rain. In the case of tiles, their porosity may play a fundamental role in accumulating contaminants. For terraces, the marginal inclination angle prevents effective washing off with first instances of rain.

Table 1. Methods and research apparatus for analysing the microbiological state of rainwater.

Indicator	Test method	Equipment
overall mesophilic and psychrophilic bacteria count	plate culture and bacteria cultured on standard nutrient media (nutritional agar) and enriched ones (agar R) ; oxygen conditions	sterilizer, autoclaves, bacteriological culture cabinet, incubator, scales
number of pathogenic bacteria - *Eschericha coli*	membrane filter technique and culture on selective and differential media; oxygen conditions	sterilizer, autoclaves, bacteriological culture cabinet, incubator, scales, a filtration kit
number of pathogenic bacteria - Enterococci (faecal streptococci)	membrane filter technique and culture on selective and differential media; oxygen conditions	sterilizer, autoclaves, bacteriological culture cabinet, incubator – 37°C, scales, a filtration kit
number of spore bacteria *Clostridium perfringens*	plate culture and cultures on nutrient, selective, and differential media; oxygen conditions	sterilizer, autoclaves, bacteriological culture cabinet, incubator – 37°C,
live and dead bacteria cell count	cytometry analysis with fluorescent dye	flow cytometer PARTEX, fluorescent dyes: Syto Green, and propidium iodide, bacteriological culture cabinet, incubator – 37°C

Table 2. Pathogenic bacteria count in rainwater – early spring (13 March 2015)

Indicator	The number of bacteria in 100 ml water sample [cfu]				
	P	B	C	O	Ż
E. coli (standard substrate)	2	0	0	0	1
E. coli (chromogenic substrate)	7	0	0	0	3
streptococcus faecalis	2	8	6	0	12
Clostridium perfingens	0	1	0	0	1

Table 3. Pathogenic bacteria count in rainwater – spring (11 May 2015)

Indicator	The number of bacteria in 100 ml water sample [cfu]				
	P	B	C	O	Ż
E. coli (standard substrate)	41	47	55	0	7
E. coli (chromogenic substrate)	54	35	2	1	57
streptococcus faecalis	7	24	47	0	7
Clostridium perfingens	0	0	0	0	0

Escherichia coli, faecal streptococci, and *C. perfingens* are microorganisms found in large amounts in human and animal faeces. The presence of these bacteria in roof-collected water may be accounted for by faeces left by birds and small mammals (mainly in the case of the terrace) [Śmigielska, 2010]. Another potential sources of faecal microorganism contaminants are wind-transported soil particles coming from organically fertilized farming land and bioaerosol from freestanding biological reactors of the water treatment plant. This is even more probable considering the fact that the points of water sample collection are located east of the abovementioned potential sources of microorganisms and the prevailing type of wind is westerly. Some studies that corroborate such hypothesis indicate that bacteria can travel inside wind-carried drops over a distance of 1 km and still remain their viability [Korzeniewska et al.., 2008, Ekstrom S. et al.., 2010**,** Ahern et al., 2007, Cho and Chwang, 2011, Polymenakou 2012]. Water collected from a galvanized sheet rooftop proves to be almost free from pathogenic microbes. This of course is due to the bactericidal effects of zinc and a relatively even surface of sheet. Furthermore, the amounts of *E. coli* obtained with classic and chromogenic media are noteworthy. In most cases more bacteria grew on the latter one (Tables 2, 3, 4).

In temperate climates the amount of bacteria is variable and depends mainly on the temperature and humidity [Niewolak and Mindrow, 2006]. Figure 1 illustrates the seasonal variation of a detected *E. coli*. The optimum temperature for growth of pathogenic microorganisms (mainly inhabited by warm-blooded organisms) is close to 37 °C. In the early spring and spring season the number of these bacteria growing in the water with tiled roofs and from the air. In the summer

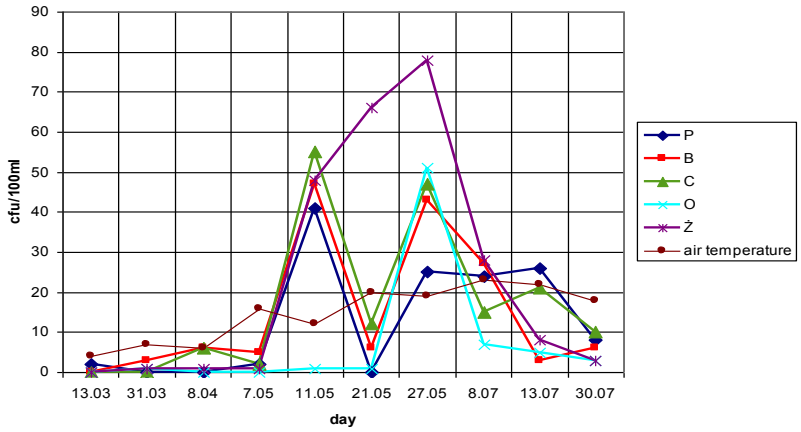

Figure 1. Changes in the number of *E. coli* bacteria during the season spring – summer

season was a decrease in the amount, despite the absence of an express temperature fluctuations. This may be due to excessive heat roofs.

An important indicator for microbial quality of water is also the overall bacteria count determined usually with the plate method. Performed studies suggest that on standard nutrient medium only some of live bacteria in a tested sample cultivate. Employing enriched agar R and extending incubation time led to significantly higher numbers of colonies (Tables 5, 6, 7).

Plate methods, however, are very time-consuming and highly error-prone, because most of live bacteria cultivate in laboratory conditions, which renders the results unreliable [Krøjgaard, 2011]. In a study of microbiology rainwater method is used Real-Time PCR (Kaushik and Balasubramanian 2012, Ahmed at al., 2014).

Table 4. Pathogenic bacteria count in rainwater – summer (13 July 2015)

Indicator	The number of bacteria in 100 ml water sample [cfu]				
	P	B	C	O	Ż
E. coli (standard substrate)	8	6	10	63	87
E. coli (chromogenic substrate)	8	25	12	8	23
streptococcus faecalis	0	55	12	63	89
Clostridium perfingens	0	0	0	0	0

Table 5. Bacteriological test results for rainwater – early spring (13 March 2015)

Water sample	Psychrophilic bacteria [cfu/ml]		Mesophilic bacteria [cfu/ml]		Flow cytometry [counts/ml]		
	Agar „A"	Agar „R"	Agar „A"	Agar „R"	SG*	JP**	Totality***
P	0	300	0	0	13250	5050	8200
B	0	900	0	0	21021	1951	19070
C	0	60	0	0	18400	1400	17000
O	630	700	0	0	11064	640	10424
Ż	78	165	0	0	1906	256	1650

Table 6. Bacteriological test results for rainwater – spring (11 May 2015)

Water sample	Psychrophilic bacteria [cfu/ml]		Mesophilic bacteria [cfu/ml]		Flow cytometry [counts/ml]		
	Agar „A"	Agar „R"	Agar „A"	Agar „R"	SG*	JP**	Totality***
P	19500	38000	2100	5500	226700	46700	180000
B	2900	289000	490	600	468000	150000	318000
C	7500	18100	1000	300	348000	58000	290000
O	1500	3700	300	400	22500	4500	18000
Ż	24300	29000	800	3000	61200	2200	59000

Table 7. Bacteriological test results for rainwater – summer (13 July 2015)

Water sample	Psychrophilic bacteria [cfu/ml]		Mesophilic bacteria [cfu/ml]		Flow cytometry [counts/ml]		
	Agar „A"	Agar „R"	Agar „A"	Agar „R"	SG*	JP**	Totality***
P	13500	16000	140	220	13000	16000	29000
B	15000	19800	256	310	43800	1200	45000
C	26100	28900	189	195	30250	1250	31500
O	1200	1870	110	271	180	700	2500
Ż	16000	23000	1780	1740	332500	118000	450500

* SG – total number of cells with nucleic acid
** JP – total number of dead cells with nucleic acid
*** Totality – total number of living cells/ml

Flow cytometry was used to assay the actual cell count. The method so far has been utilized mainly in laboratory diagnostics for assays of apoptopic and unusual (carcinogenic) bacteria counts. Indications of the numbers of live bacteria obtained with cytometry for each tested water and for each collection period were even a few times higher. The largest differences were revealed for early spring season and the most similar for spring. This is probably due to the fact that the activity of most micro-temperate (including bacteria) are significantly reduced at low temperatures (0–10 °C) and their proliferation on the medium is not very effective. Flow cytometry also allows you to mark those cells that reproduce in the laboratory [Hammes F. et al., 2008; Steinberg M. et al., 2013]. Much smaller amounts of mesophilic bacteria in relation to psychrophilic result of moderate temperatures, even in the summer.

Due to the above normal psychrophilic and mesophilic bacteria, including faecal waste, tested water from rainfall is not suitable for drinking (Dz.U of 25 February, item. 257). Also, in the agricultural use of water with a relatively high content of coliforms and faecal streptococci and C. perfingens, it poses a threat to human health (Dz.U of 25 February, item. 257)

There are already many developed methods of disinfection of rainwater for household and agriculture. Used solar panels - it is possible to thermal inactivation of pathogenic bacteria (Amin and Han, 2009, 2011), as well as membrane filtration downstream such waters from the surface of the roof (Naddeo at al., 2013) or sand filters (Neto at al, 2012). Chemical methods such as chlorination (Neto at al, 2012), the use of silver ions (Navaz et al., 2012).

CONCLUSIONS

1. Rainwater at the time going through the outside air is contaminated microbiologically, which is associated with the presence of bioaerosol.
2. Rainwater collected from roofs have a large load of microbiological pollution, including dangerous to the health of faecal bacteria.
3. The presence of bacteria of faecal origin precludes the use of rainwater for drinking and watering plants whose fruits are in contact with the ground, and household water collected from roofs can serve only that for the purpose of cleaning in the bypass.
4. It is possible to use rainwater in horticulture agriculture, in industry and for the purpose of cleaning, preferably after its disinfection (physical or chemical).
5. It is advisable to use alternative methods for determining the quality and quantity of microorganisms in order to obtain more reliable results of these assays.

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