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Editorial

In this issue of the *International Journal of Sanitary Engineering Research* the readers can find four articles with the various interesting topics. M. Cvetković and B. Kompare studied the influence of ballast water on the marine ecosystems. This topic is of high interest for the Slovenia too because of the port Koper. K. Godič Torkar and R. Fink are discussing the potential applications of rapid microbiological methods for detection of antibiotic residues in wastewater, surface and well water. A. Ovca and his colleagues investigated the efficiency of thermal insulating bags during transport of cooled food items.

The last topic which is also discussed here is sanitary – technical and hygienic conditions of Slovenian kindergartens with the improvement proposals. K. Kocjan Žgajnar, A. Galičič, U. Zoran, L. Pajek and M. Dovjak investigated above mentioned conditions in 35 playrooms in 16 kindergartens. According to the presented results in many playrooms sanitary – technical and hygienic conditions do not fulfil regulation demands. Namely irregularities are many related to improper installation of final coverings, to low parapet heights and total opening area of windows, poor hygienic conditions, and selection of materials that may present health risks. The measures proposed for the improvement are beside others also changing the PVC windows frames with wooden ones and providing proper size of total window surface.

Slovenian government issued three tenders for co-financing the energy renovation of the public buildings owned by the municipalities. Through this action many kindergartens is to be renovated. The renovation includes changing the windows and doors, thermal insulation of the facades and roofs, renovation of the heating systems. This means that this is proper time that municipalities, designers and other stakeholders take into the account proposed measures in order to improve sanitary – technical conditions in their kindergartens. In many kindergartens the existing windows (with frames from various materials) will be replaced with the energy efficient ones with wooden frames. Thermal insulation of the facades and ceilings includes use of the glass wool and rock wool. Installation of the polystyrene thermal insulation increase risk of fire and decrease safety of the pupils and children. After the energy renovation of the buildings another problem will appear. Namely with the renovation of the building envelope the natural ventilation is almost prevented. The installation of the ventilation systems is not eligible costs and is not installed. During the heating season the air quality in playrooms will be decreased. There we are faced with the paradox that energy renovation of the buildings has negative influence on the sanitary conditions in the buildings. There are several possibilities available to improve air quality in the playrooms. One of the simplest is ongoing ventilation with the partly opened windows. In this case the energy losses are too high. The second most appropriate possibility is periodic natural ventilation with the short term full opened windows. The kindergarten teachers should be proper educated how to keep proper air quality in their playrooms not to increase energy losses. Therefore the researches in this topic should continue or repeat their investigations after the energy renovation of the kindergartens is finished.

Sincerely,

Editor-in-chief
Janez Petek

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Content

Original scientific article

Analysis of sanitary--technical and hygienic conditions of Slovenian kindergartens and proposed measures

Katarina **KACJAN ŽGAJNAR**[1], An **GALIČIČ**, Urška **ZORAN**, Luka **PAJEK**, Mateja **DOVJAK**[2*]

ABSTRACT

Most children in early childhood spend approximately one third of the day in kindergartens. Therefore, it is essential that their stay in an educational institution is comfortable and without adverse health effects. Statistical data over the last decade show that the amount of enrolled children in kindergartens has increased. The problem of overcrowded kindergartens is usually solved by changing the existing norms for class sizes, or with alternative spaces that do not serve the original purpose. According to EU Directives, kindergartens are among building facilities that have to be renovated. The purpose of the paper was to analyse sanitary-technical and hygienic conditions of the selected playrooms of kindergartens and to define measures. Real-state conditions were evaluated in 35 playrooms of 16 kindergartens in the Central Slovenian region. The main emphasis was on the used materials, their cleaning capability, room acoustics, natural daylight, safety and comfort issues, as well on their possible adverse health effects. The combination of observation, measurements and calculations of reverberation time, and comparison with regulation demands was carried out. In many playrooms sanitary-technical and hygienic conditions did not fulfil regulation demands. Irregularities are mainly related to improper installation of final coverings, to low parapet heights and total opening area of windows, poor hygienic conditions, and selection of materials that may present health risks. Calculated and measured reverberation times deviated from optimal values.

Key words: playrooms, sanitary-technical conditions, hygiene, noise, measures

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INTRODUCTION

In Slovenia, children are included in the system of pre-primary education (i.e. kindergartens, day-care centres, preschool) from the end of the maternity leave (i.e. 11 month old) till starting compulsory education [1]. According to preschool legislation, learning standards and guidelines [2-5], the maximum kindergarten class sizes are from 14 to 24 children, depending on the child age, special needs and disabilities. Kindergartens present biological, physical and social environment where a child usually spends approximately one-third of the day. The environment of kindergarten has a strong interactive influence on a child [6], so it is very important to assure a high level of safety without any health risks.

Children represent a specific population group, mainly due to their lower body weight, higher activity levels and age-related behavioural characteristics. In the childhood, the immune system is not fully developed, so children are more susceptible to environmental influences [7,8] than adults. Moreover, entering kindergarten also presents a physiological health risk for a child (i.e. stress). Epidemiological data indicate an increased morbidity among children in the first months of visiting kindergartens [9].

Statistical data of the Republic of Slovenia showed that in the school year 2012/13 almost 77 % of all children of proper age were enrolled in kindergartens. The number of enrolled children in kindergartens increased from 70 % in school year 2008/2009 to 77 % in school year 2012/13 [10]. The EU benchmark on pre-school participation stipulates that by 2020 at least 95 % of children between the age of 4 and the starting age of compulsory education should participate in early childhood education [10-12]. Despite large amount of children enrolled in early childhood education system, many existing buildings do not fulfil pre-school standards and guidelines. Moreover, the problem of overcrowded kindergartens is usually solved by changing existing norms for class sizes, or with alternative spaces that usually do not serve the original purpose (i.e. containers, mobile houses). For example, the average age of the selected kindergarten buildings in the Central Slovenian region is 1978. According to EU Directives [13-15], kindergartens are among the building facilities, which have to be renovated.

Current studies on kindergartens and health issues are mainly focused on chemical risks, i.e. chemical pollutants in indoor air [16,17], metal contamination [18,19], radon emission sources [20]; biological risks, i.e. transmission of biological agents [21-23], microbiological quality and safety of food [24] as well on physical risks, i.e. noise [25-28]. The review by Le Cann et al. [29] was taking a broad approach to the indoor environment and including chemical, microbial, physical and social aspects.

Noise as a physical health risk in kindergartens presents well researched topic [25-27]. McAllister et al. [25] studied children's exposure to background noise at the ears during a normal day in three day-care centres in Linköping, Sweden. Chatzakis et al. [26] performed measurements of noise levels in occupied and unoccupied classrooms

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in overall ten kindergartens in the city of Heraklion, Crete, Greece. Sjödin et al. [27] carried out an investigation on 101 employees at 17 preschools in Umeå county, Sweden. Voss [28] investigated the correlation between the reverberation time (that is the time that would be required for the sound pressure level in the enclosure to decrease by 60 decibels after the source has been stopped) and the resulting room noise level, as well as the correlation between the one-hour room noise levels and the number of children present in day-care centres, Denmark. Kovačič and Kacjan Žgajnar [30] and Kacjan Žgajnar et al. [31,32] measured equivalent and impulse levels of noise in kindergartens in Ljubljana, Slovenia. The results from the above studies often exceeded the permissible noise levels for working and living environments [33-35]. Based on the results by Voss [28], three main factors for effective reduction of noise levels in day-care centres were defined, i.e. physical surroundings (buildings, rooms, etc.), the number of children, and social behaviour. Other guidances in schools also include acoustic [36] as well as educational measures [37]. The effectiveness of measures for the reduction of noise levels was evaluated in a few studies. Gerhardsson and Nilsson [38] studied noise-related problems in personnel at Swedish day-care centres before and after acoustical treatment. L'Espérance et al. [39] performed measures of noise levels, reverberation time and the surface radiation factor at 40 locations in 20 day-care centres in Quebec. Installing an acoustic ceiling and adding a band of acoustic panels at the top of the walls reduced noise levels on average by 6 to 7 dBA [39] and 2 to 6 dB [38]. There even exist defined recommendations for the reduction of noise levels in kindergartens, but there is still a large gap between recommended implementation and execution. Studies dealing with overall sanitary-technical and hygienic issues of kindergarten playrooms are rare. However, there are no defined measures important for building design and renovation.

Studies dealing with overall sanitary-technical and hygienic issues of kindergarten playrooms are rare. However, there are no defined measures important for building design and renovation.

The purpose of our paper is to analyse sanitary-technical and hygienic conditions of selective playrooms of kindergartens in the Central Slovenian region. The main emphasis was on the used materials, their cleaning capability, room acoustics, natural daylight, safety and comfort issues, as well on their possible adverse health effects. The main findings will be compared with current regulations and guidelines. Based on the comparison, measures important for building design stage, construction and renovation will be prepared.

STATISTICAL BACKGROUND: SLOVENIA, EU

In Slovenia, in the school year 2012/13, almost 77 % of all children of the proper age are enrolled in kindergartens [10]. In the school year 2012/13, 938 kindergartens and their units were providing pre-school education; this is 16 more than in the previous school year [10]. The majority (95 %) of kindergartens are public; only 50 or 5 % are private. In the school year 2012/13 the number of children enrolled in kindergartens increased by 2.3 % over the previous school year, but the growth is not as high as in the school years 2008/09 to 2011/12,

when the annual growth rate was around 7 %. Slightly more than 83,000 children were enrolled in kindergartens and in child-minders' families, which is 76.7 % of all children of the proper age. Compared to the previous school year, the number of children particularly increased in the second age period (children from the age of 3 up to entering basic school), which represented a 3.3 % increase in enrolment, so that the share now stands at almost 90 %. In the first age period (children up to 3 years) the number of children increased only slightly. Kindergartens now include more than half of the children in this age group. Slightly more than 38,500 4- and 5-year-olds are enrolled in pre-school education, which is 93.3 % of all children of that age [10]. In the school year 2012/13 pre-school care and education in kindergartens is provided by about 10,453 professional staff, of whom 4,986 are educators and 5,467 assistant educators. There are 2 % men – mostly assistant educators – among professional staff. There are on average 8.1 children per educator and assistant educator; in the first age period 6.2 children and in the second age period 9.4 children [10].

According to EU statistics [11,12], there was a significant rise in children attending pre-primary education, from 85.6 % in 2000 to 92.5 % in 2009 (between the age of 4 and the start of compulsory primary education). In Belgium, Spain, France, Italy and the Netherlands, all children are enrolled at the age of 4 until the start of (compulsory) primary education, while Germany, Cyprus, Latvia, Lithuania, Austria, Poland, Portugal, Romania, Slovenia, Finland and Sweden have seen significant increases in participation over the period. In general, in the EU Member states, high levels of children attending pre-primary education correspond with high employment rates of women [11,12].

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METHODS

Analysis of sanitary-technical and hygienic conditions was performed from March to May 2013 in 35 playrooms (for children age from 3 to 6 years) of 16 kindergartens in the Central Slovenian region. General characteristics of playrooms and population group are described in Table 1. According to the required demands [3] the main observed groups of sanitary-technical and hygienic parameters were: material type (floor, wall, ceiling covering), cleaning capability (installation of cove fillet, washable wall covering), safety (parapet height, protection of radiator surfaces), natural daylight (window-to-floor ratio), comfort (installation of wall covering up to 1.2 m high, thermal properties-warm/cool feeling to touch), basic hygienic conditions (dust, curtains), room acoustics (reverberation time). Real-state conditions were evaluated according to EU and SI legislation. Possible adverse health effects were defined according to reviewed studies.

Room acoustics were evaluated with calculated and measured reverberation times. Reverberation time was calculated with Sabine formula Eq. (1) [40-42], Eyring formula Eq. (2) [41-43] and Millington-Sette formula Eq. (3) [44-46].

$$T_S = \frac{0.163\,V}{A+4\,mV} \quad (1)$$

where T_S is calculated reverberation time with Sabine formula [s], V is the volume of the room [m^3], A is the sum of the surface areas of the room multiplied by their respective absorption coefficients at a given frequency and m is the absorption coefficient as a function of air absorption and frequency [m^{-1}]. Sabine formula (Eq. 1) should be used for rooms with volumes less than 200 m^3 and a reasonable distribution of sound and lower sound absorption (absorption coefficient less than 0.2) [41,42].

$$T_{Ey} = \frac{0.163\,V}{-S\ln(1-\bar{\alpha})+4mV} \quad (2)$$

where T_{Ey} is calculated reverberation time with Eyring formula [s], where V is the volume of the room [m^3], S is the total surface area of the room in [m^2], $\bar{\alpha}$ is average absorption coefficient [-] and m is the absorption coefficient as a function of air absorption and frequency [m^{-1}]. Eyring formula (Eq. 2) should be used for rooms with higher sound absorption (absorption coefficient more than 0.2) [41,42].

$$T_{M-S} = \frac{0,16\,V}{-\sum_i S_i ln(1-\alpha_1)} \quad (3)$$

where T_{M-S} is calculated reverberation time with Millington-Sette formula [s], V is the volume of the room [m^3], α_1 is the sound absorption coefficient as sub-area S_i. Millington-Sette formula [44-46] should be used when the materials of a room have a wide variety of absorption coefficients [46]. Absorption coefficients at 500 Hz (relevant for child voice, baby cry, unoccupied room [47]) were selected from the relevant literature [48]. For the calculation it was assumed that the rooms were unoccupied.

Measurements of reverberation time were performed in two typical playrooms (playroom No. 28, located in kindergarten M; playroom No. 33 located in kindergarten O). Measurements were conducted according to the standards [49,50]. Reverberation time was measured with calibrated modular precision sound analyser type 2260 Investigator, manufacturer Brüel and Kjaer.

The observed sanitary-technical and hygienic conditions were evaluated according to the Rules on the criteria and the minimum technical requirements for space and equipment of kindergartens [3], Rules on the acoustic insulation in buildings [41], TSG-1-005:2012 [42], Rules on the protection of workers from the risks related to exposure to noise at work [33], Rules on the ventilation and air-conditioning of buildings [51], Regulation (EU) No 305/2011 [15] of the European Parliament and of the Council of 9 March 2011 laying down harmonised conditions for the marketing of construction products and repealing Council Directive 89/106/EEC.

Calculated and measured reverberation times were compared to optimal levels for classrooms according to [42].

$$T_{opt} = 0{,}32\ log\ V - 0{,}17 \qquad (4)$$

where T_{opt} is calculated optimal reverberation time [s] and V is the volume of the room [m^3].

RESULTS AND DISCUSSION

Design of active spaces

According to regulation demands [3], all active spaces in kindergarten have to be functionally designed according to child age. The position of active spaces must be transparent and directly connected to the central area. The plan of the playroom should be dynamic with minimum surface area 40 m^2.

The examined 35 playrooms were functionally designed according to child age group 3-6 years with transparent and direct connection to the central area. All plans were dynamic, but the surface areas of 13 playrooms (No. 1, No. 3, No. 7-9, No. 16, No. 22, No. 25, No. 26, No. 28 and No. 30-32) were less than 40 m^2; surface areas of the following 4 playrooms were less than 35 m^2 (No. 1, No. 3, No. 7, No. 9).

Table 1:
Analysed 35 playrooms of 16 kindergartens (A-P) in the Central Slovenian region and population group.

Kindergarten	Playroom No.	Construction year [yr]	No. of children [ ]	No. of professional staff [ ]
A	1	1952	21	2
	2		23	3
B	3	1982	24	2
C	4	1973	19	2
	5		24	2
	6		23	2
D	7	1976	21	2
	8		20	2
	9		20	2
	10		21	3
	11		23	2
E	12	1976	21	3
F	13	1979	19	2
	14		20	3
G	15	2005	22	2
H	16	2013	24	2
I	17	1971	19	2
	18		24	2
J	19	1972	19	2
	20		23	2
	21		21	2
	22		18	2
	23		19	2
K	24	1963	21	2
	25		19	3
	26		21	2
L	27	1906	20	3
M	28	1979	22	4
N	29	1976	20	2
	30		21	2
	31		22	2
	32		21	2
O	33	1976	19	2
P	34	2012	24	2
	35		23	2

Sanitary-technical conditions

Results of the evaluation of sanitary-technical conditions of the 35 playrooms in 16 kindergartens in the Central Slovenian region are presented in Table 2. Fulfilment of regulated demands is marked with + (evaluated real-state condition fulfilled regulation demand) or – (evaluated real-state condition did not fulfil regulation demand).

Used materials in playrooms and their health issues

Regulation (EU) No. 305/2011 [15] of the European Parliament and of the Council of 9 March 2011 laying down harmonised conditions for the marketing of construction products and repealing Council Directive 89/106/EEC define basic requirements for construction works and construction products (materials) that have to be fulfilled throughout the whole life cycle of a building. The construction works as a whole and their separate parts must be fit for their intended use, taking into account in particular the health and safety of persons involved throughout the life cycle of the works. Some of the main issues of the Regulation are hygiene, health and environment; safety and accessibility in use; protection against noise explicitly defined in basic requirements No. 3, No. 4 and No. 5. The Regulation [15] shall be binding in its entirety and directly applicable in all Member States, as well as harmonized with their horizontal and vertical legal framework.

The majority of materials used in playrooms were wood (particle boards, plywood), parquet, linoleum, paint, glass, washable upholstery cushions, foam, cork, paper, fabric, and PVC (window frames). Most of used materials are harmless, but according to epidemiological studies some of them may present health risks.

Basic requirement No. 3 – Hygiene, health and the environment [15], relevant for used materials, demands that construction works must be designed and built in such way that throughout their life cycle they will not be a threat to the hygiene or health and safety of workers, occupants or neighbours, nor have an exceedingly high impact, over their entire life cycle, on the environmental quality or on the climate during their construction, use and demolition. This basic requirement is harmonized with the Rules on the criteria and the minimum technical requirements for space and equipment of kindergartens [3] which defines that all constructional and installation products must be environmentally friendly without health risks.

The majority of materials used in playrooms were wood (particle boards, plywood), parquet, linoleum, paint, glass, washable upholstery cushions, foam, cork, paper, fabric, and PVC (window frames). Most of used materials are harmless, but according to epidemiological studies some of them may present health risks [52-59]. The literature review [53,54] showed that wooden construction products and furniture as well as paints, adhesives, varnishes, floor finishes, disinfectants, cleaning agents and other household products present the main indoor sources of formaldehyde. Paints, varnishes and cleaning agents may be the source of volatile organic compounds (VOCs) [55-57]. Linoleum has been shown to emit a series of aldehydes and fatty acids as major VOCs [60]. Various studies [55,56,58,59] have indicated that numerous indoor sources and insufficient ventilation often result in higher formaldehyde and VOCs levels and may cause adverse health effects. Wieslander et al. [56] indicated that exposure to chemical emissions from

Table 2:
Evaluation of sanitary-technical conditions in 35 playrooms of 16 kindergartens, Central Slovenian region.

Playroom/ Observed parameter	Material			Cleaning capability		Safety issues		Natural daylight	Comfort	Basic hygienic conditions	
	Floor	Wall	Ceiling	Cove fillet	Washable wall covering	Parapet height [m]	Protection of radiator surfaces	Window-to-floor area ratio [%]	Installation of wall covering up to 1.2 m high. Thermal properties- warm/cool feeling to touch	Dusty shelves	Curtain
1*	parquet	paint, wood	paint	-	+	0.65	+	22.39	+	+	+
2*	laminate	paint	paint	-	-	1.00**	+	36.77	-	+	+
3	parquet	paint, wood	paint	-	-	0.61	+	34.92	-	-	+
4	linoleum	paint	wood	+	+	0.54	+	35.01	-	-	-
5	linoleum	paint	wood	+	+	0.54	+	19.48	-	-	+
6	linoleum	paint	wood	-	+	0.60	+	18.74	+	-	+
7	linoleum	paint	Armstrong gypsum board	-	+	0.80**	-	33.12	-	+	+
8	linoleum	paint	Armstrong gypsum board	-	+	0.80**	-	28.15	-	+	+
9	linoleum	paint	Armstrong gypsum board	-	+	0.80**	-	36.08	-	+	+
10	linoleum	paint	Armstrong gypsum board	+	+	0.90	-	23.46	-	+	+
11	linoleum	paint	Armstrong gypsum board	+	+	0.90	-	25.27	-	+	+
12	linoleum	paint, wood	paint	-	+	0.50	+	36.29	+	+	+
13	linoleum	paint, wood	paint	+	+	0.40	+	21.80	+	+	-
14	linoleum	paint, wood	paint	+	+	0.52	+	21.02	+	+	-
15	laminate	paint	paint	-	+	0.55	+	18.49	-	-	+
16	linoleum	paint	paint	-	-	0.00	-	44.43	-	+	+
17	parquet	paint	Armstrong gypsum board	-	+	0.60	-	25.09	-	-	+
18	parquet	paint	paint	-	+	0.60	-	31.74	-	+	+
19	parquet	paint	paint	-	+	0.89**	+	25.96	-	+	+
20	parquet	paint	paint	-	+	0.89**	+	25.01	-	+	+
21	parquet	paint	paint	-	+	0.89**	+	26.40	-	+	+
22	parquet	paint	paint	-	+	0.85**	-	35.39	-	+	+
23	parquet	paint	paint	-	+	0.85**	-	21.21	-	+	+
24	linoleum	paint, wood	paint	+	+	0.74**	-	31.62	+	-	+
25	linoleum	paint, wood	paint	+	+	0.73**	-	41.63	+	-	+
26	linoleum	paint, wood	paint	+	+	0.75**	+	22.77	+	-	-
27*	parquet	paint, wood	paint	-	+	0.60	+	9.09	+	+	+
28	linoleum	paint, wood	paint	-	+	0.55	+	27.44	+	+	-
29	linoleum	paint, wood	paint	+	+	0.90**	-	39.92	+	-	-
30	linoleum	paint, wood	wood	-	+	0.90**	-	38.42	+	-	-
31	linoleum	paint, wood	wood, steel (I beam)	-	+	0.90**	-	46.24	+	-	-
32	linoleum	paint, wood,	wood, steel (I beam)	+	+	0.86**	-	39.19	+	-	-
33	linoleum	Wood, paint	Armstrong gypsum board	+	-	0.60	-	37.38	-	+	+
34	rubber	paint, rubber	paint	-	+	0.00	-	35.67	+	+	+
35	rubber	paint, rubber	paint	+	+	0.00	-	26.24	+	+	+

*Building was not intended for educational purposes; **Playroom is located in the first floor.

indoor paint is related to asthma, and that some VOCs may cause inflammatory reactions in the airways. Results by Norback et al. [55] suggested that indoor VOCs and formaldehyde may cause asthma-like symptoms. Used PVC materials for window frames (installed in playrooms No. 1-2, No. 4-11, No. 15, No. 17-23, No. 29-32; representing 62.9 % of total playrooms) also may present an important cause of health concerns. The literature review on health concerns associated with PVC building materials concluded that PVC building material presents an important emission source of phthalates that may have adverse health effects [61-64]. Most exposed population is young children and certain groups of adults during occupational exposure. Various studies [63,64] have indicated that these chemicals may be endocrine disruptors and may lead to the development of asthma, allergies, or related respiratory effects. Additionally, PVC window frames are usually wider than wooden ones, and provide less amount of daylight [65]. All building materials, where there exist scientific facts for adverse health effects, should be fully eliminated, especially in buildings occupied with sensitive population group.

The literature review on health concerns associated with PVC building materials concluded that PVC building material presents an important emission source of phthalates that may have adverse health effects.

All building materials, where there exist scientific facts for adverse health effects, should be fully eliminated, especially in buildings occupied with sensitive population group.

Hygienic and comfort issues

Rules on the criteria and the minimum technical requirements for space and equipment of kindergartens [3] define that all floor coverings must be solid, non-slip and should allow effective wet cleaning. Additionally, the floor-wall junctions (cove fillets) must be installed to assure easy and effective wet cleaning [3]. Floor coverings in all playrooms met the criteria, except floor coverings in playrooms No. 1-3, No. 15, No. 17-23 and No. 27 that were covered with laminate or parquet. Laminate and parquet do not allow effective wet cleaning, disinfection and they may absorb water. Floor-wall junctions were properly installed in 13 playrooms; among them there were 12 playrooms covered with linoleum and 1 playroom with rubber. Floor-wall junctions were not properly installed in playrooms where floors were mainly covered with parquet and laminate.

Wall coverings up to 1.2 m high must be washable and made from materials that are pleasant to touch and feel warm, and with proper abrasion resistance [3]. Wall coverings were washable in 31 playrooms where wall coverings were made from waterproof paint or wooden panels. Wall coverings in 4 playrooms (No. 2, No. 3, No. 16 and No. 33) did not fulfil the regulated demands, because they were made from non-waterproof paint. Wall coverings in 16 playrooms were made from materials that are pleasant to touch and feel warm (wood). Wall coverings in 19 playrooms did not fulfil the regulated demands; they were covered with wall paint.

Effective and regular cleaning contributes significantly to the prevention and control of infectious diseases. Dust deposition on playroom surfaces may lead to bacterial growth [66]. Study [67] found a correlation between the content of organic dust in carpets and the appearance of symptoms of sick building syndrome (SBS). Additionaly, Gyntelberg et al. [68] found a significant correlation between the prevalence of Gram-

negative bacteria in the indoor dust and symptoms such as fatigue, heavy-headedness, headache, dizziness and lack of ability to concentrate, and symptoms from the mucus membrane of the upper respiratory tract. Dust mites and their residue found in beds, pillows, carpets, and furniture surfaces cause allergies [69]. Basic hygienic conditions in many playrooms were poor. 13 playrooms from overall 35 had dust deposited on the top of shelves, 9 playrooms from overall 35 had window curtains.

Safety issues

In all playrooms high level of safety must be provided. Basic requirement No. 4 – Safety and accessibility in use [15], requests that the construction works must be designed and built in such a way that they do not present unacceptable risks of accidents or damage in service or in operation, such as slipping, falling, collision, burns, electrocution, injury from explosion and burglaries. In particular, construction works must be designed and built taking into consideration accessibility and use for disabled persons. Therefore, Rules on the criteria and the minimum technical requirements for space and equipment of kindergartens [3] define that all floor coverings must be solid and non-slip. All wall corners up to 1.2 m must be secured with rounded corner profiles. All the furniture corners and edges must be smooth and free of sharp edges. Heavy and high furniture must be screwed to the floor and to the wall [3]. All playrooms fulfilled the safety demands.

The required maximum height of window parapets in playrooms that are located at the ground floor is 0.60 m above the ground, and minimum 0.90 m for playroom located in the first floor. All windows have to assure high level of safety and prevent falling out of windows [3]. Parapets in 4 out of 18 playrooms located at the ground floor did not meet the criteria; parapets in 13 out of 17 playrooms located in the first (or higher) floor did not meet the criteria. Overall 48.6 % of playrooms did not comply with conditions.

Natural daylight

External environment has stimulative effect on the human body and mind. Daylight provides quality lighting, stimulates sense of sight and is an important communication between the internal and external space [65]. Several researches showed connection between natural daylight and improved learning outcomes in schools. Other positive effects of natural daylight are: better illumination, visual stimulation, improved concentration and responsiveness, faster learning, less illnesses, less dental decay (cavities), improved eyesight, increased body growth and improved immune system [65,70-72].

Natural daylight has to be provided in all playrooms. The depth of the playroom should not be more than 2.5 times height level (from the ground to the upper edge of the window); otherwise the playroom must be illuminated from two room sides. The total opening area of windows must not be less than 1/5th the floor area of the room [3]. 25 playrooms fulfilled the criteria for the depth of the playrooms. 10 playrooms

Dust deposition on playroom surfaces may lead to bacterial growth. Study found a correlation between the content of organic dust in carpets and the appearance of symptoms of sick building syndrome (SBS).

did not meet the criteria and had room depths more than 2.5 times the height level, only 4 of them were illuminated from two room sides. Playroom No. 16 was illuminated from two room sides and had ratio 3.76. Playroom No. 34 had the worst ratio 2.80 and was not illuminated from two room sides. 31 playrooms had the total opening area over 1/5 of the floor area of the room, 14 of them had the total opening area over 1/3th of the floor area of the room (No. 2-4, No. 9, No. 12, No. 16, No. 22, No. 25 and No. 29-34). Playrooms No. 5, No. 6, No. 15 and No. 27 did not meet the criteria for the total opening area of windows. Playroom No. 27 had the total opening area of windows 9.09 % (less than 1/11th of the floor area).

Clean, double glazed windows transmit about 70 % of daylight. On the other hand, triple glazed windows, in an effort to improve thermal insulation, transmit barely about 50 % of daylight [65]. After observing several playrooms, we found out that 24 % of them had triple glazed windows. This type of glazing was noticed in playrooms with replaced windows, in an attempt to reduce energy losses, and in playrooms of the newly build kindergartens. As we know, triple glazed windows transmit less natural daylight than double glazed windows [65]. Additionally, in evaluated playrooms daylight penetration was often distracted by inappropriate use of shading system or even by various items attached to the window (shaped paper, drawings etc.).

Analyses of reverberation time

Basic requirement No. 5 – Protection against noise, requests that construction works must be designed and built in such a way that noise perceived by the occupants or people nearby is kept to a level that will not threaten their health and will allow them to sleep, rest and work in satisfactory conditions. Rules on the criteria and the minimum technical requirements for space and equipment of kindergartens [3] state that floor, wall and ceiling coverings must be made of materials that reduce noise. Rules on the acoustic insulation in buildings [41] and Technical guidelines for protection against noise in buildings [42] define that protection against noise in buildings must provide actions against noise produced outside and/or in a building, actions against direct sound transmission (through air and structures), actions against equipment noise and actions against reverberation sound. Protection against reverberation noise has to be ensured by proper installation of sound absorptive surface elements, taking into account the size and shape of the space.

The reverberation time was measured in two playrooms, No. 28 and 33, and compared to calculated and optimal values (Figure 1).

Most playrooms had calculated reverberation time more than two times higher than optimal time. Connection (trend) between all the calculated values is very similar, with lowest values calculated with Millington-Sette formula, middle values calculated with Eyring and higher values with Sabine formula. For most of the playrooms (except playrooms No. 4 and No. 17) reverberation times that were calculated with the Sabine

formula are more accurate (absorption coefficients less than 0.2). It can be observed that the reverberation time values calculated using the Sabine, Eyring and Millington-Sette formulae significantly deviate from the measured values. Similar conclusions were found in the study by Neubauer and Kostek [46]. To compare the measured and calculated reverberation times additional analysis is needed.

Measurements of reverberation time were made in the frequency range from 100 to 8000 Hz (Figure 2). Playroom No. 28 had better sound absorption at high frequencies and worse at low frequencies, and vice versa in playroom No. 33. The main reason for this is in sound absorption of materials that depend on the frequency level. Some materials are better sound absorbers at low frequencies and some at high. That is why we have to be careful with the selection of materials for kindergartens. The data [47] showed that baby cries have a basic frequency of around 500 Hz. Child speech ranges from 250-400 Hz, adult females tend to speak at around 200 Hz on average, and adult males around 125 Hz. The average measured reverberation times for typical frequencies for child voice in playrooms (250 to 500 Hz) were 0.62 s for playroom No. 28 and 0.63 s for playroom No. 33 (optimal value 0.50 s).

Figure 1:
Calculated, measured and optimal reverberation times [s] in 35 playrooms of 16 kindergartens.

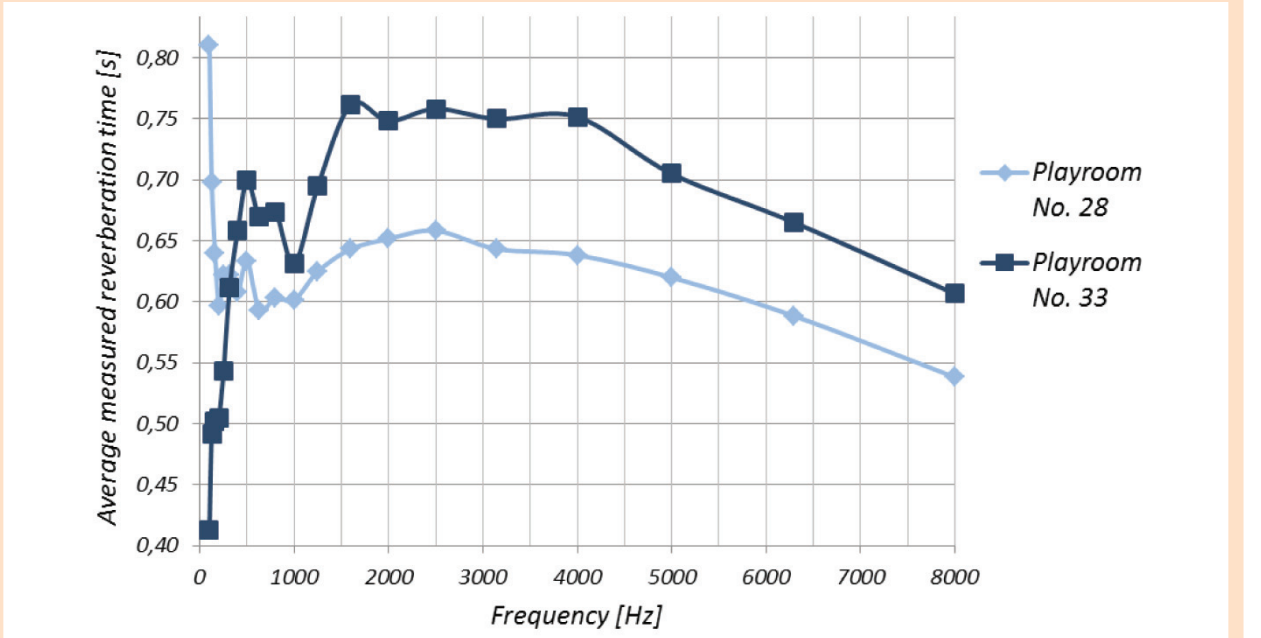

Figure 2:
Average measured reverberation time [s] in the frequency range from 100 to 8000 Hz in playroom No. 28 and playroom No. 33.

MEASURES ON THE LEVEL OF SANITARY-TECHNICAL AND HYGIENIC ISSUES WITH NOISE PREVENTION

Analysis of 35 playrooms in 16 kindergartens in Central Slovenian region showed that in many playrooms sanitary-technical and hygienic conditions did not fulfil the regulation demands. Based on the evaluation of real-time conditions, measures were prepared. They include actions on the level of sanitary-technical and hygienic improvements, implication of health and environment friendly building materials as well as measures for effective noise prevention.

Sanitary-technical improvements of analysed playrooms:

- removal of all materials where there exists scientific proof of adverse health concerns (i.e. PVC materials), replacement with health and environment friendly alternatives (i.e. PVC window frames for wooden window frames),
- installation of washable wall coverings that are zero VOC and non-toxic,
- wall coverings have to be made from materials that are pleasant to touch and feel warm,
- installation of wall-floor cove fillets and safety covers for room radiators in all playrooms where they were missing,
- repair of parapet heights or installation of safety window fences, safety windows,
- providing natural daylight with proper size of total opening area of windows (not less than 1/5th the floor area of the room),
- regular effective cleaning and maintenance.

Noise prevention and control in analysed playrooms:

- assuring optimal room acoustics with technical measures, including installation of sound acoustic elements that are hygienically adequate.

For complete noise protection it is necessary to implement technical measures defined by TSG-1-005:2012 [42] that include protection against outside noise, direct sound transmission through structures, equipment noise and reverberation sound. In kindergartens it is very difficult to meet the requirement for optimum reverberation time and at the same time avoid materials that are hygienically questionable (i.e. fabric curtain). Therefore, the selection of materials with good sound absorption and hygienically appropriate needs special attention. Organizational measures include implementation of legislation on the level of noise protection of all users (children, staff), introduction of permanent health education of teachers, parents and children, use of less noisy toys, raising the awareness in general public, state and local authorities. Alternative measures include introduction of more quiet activities in regular work day, relaxation.

All listed measures are not important only for the evaluated kindergartens, but also for the design, construction and renovation of buildings. At the level of the design, all basic bioclimatic principles presented in Krainer et al. [73] have to be implemented. Building envelope and constructional complexes have to allow optimal regulation of thermal and daylight fluxes as well as assure proper sound insulation. Holistic approach is necessary for solving problems in the first stages of design. In particular, buildings must be designed and built taking into consideration accessibility and use for disabled persons (physical, visual, hearing, mental and cognitive disability) [15, 74-76]. Sanitary-technical conditions of playrooms will be further on analysed in relation to the whole building concept. Our research areas will include thermal comfort, noise, and indoor air quality issues. All the above stated is necessary to assure comfort and healthy indoor environment for children and staff.

Holistic approach is necessary for solving problems in the first stages of design.

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Original scientific article

The efficiency of thermal insulating bags during domestic transport of chilled food items

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ABSTRACT

It has been demonstrated that temperature control is critical in the last three steps in the cold chain including transport between retail and the consumer's home. The aim of this study is therefore to evaluate the efficiency of thermal insulating bags meant to transport frozen foods from stores to consumers' homes also in case of transporting chilled foods. The efficiency of three insulating and one typical PVC bag as a control is evaluated. Evaluation is done at different internal (related to the load of the bag) and external (related to the outside temperature) conditions, also taking their price into consideration. During evaluation, measurements of test objects' internal temperature were executed at five-minute intervals with a Testo 177-T4 data logger. The measurements reveal variations of the test objects' internal temperature in accordance with air temperature outside the bag and the degree of load in the bag. The evaluated insulating bags are not efficient enough to preserve appropriate temperature environments for chilled food items under experimental conditions. There was also no confirmation of any significant impact of insulating bag purchase price.

Key words: Food safety, food transport, cold chain, insulating bags

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INTRODUCTION

Refrigeration is one of the most widely practiced methods of preserving the quality and safety of foodstuffs. Maintaining a cold chain is an important preventive step for ensuring food safety, and temperature is one of key parameters effecting growth of microorganisms and their survival in food. Therefore, in order to provide safe food at high quality, attention must be paid to every aspect of the cold chain from production to consumption. It is important that the process of maintaining the cold chain does not end with the retailer. Considering the potential microbiological risk presented by perishable food items, maintaining the cold chain should continue up to and within a consumer's home.

Considering the potential microbiological risk presented by perishable food items, maintaining the cold chain should continue up to and within a consumer's home.

Transfer points are well known problem areas for temperature mishandling and refer to points in the cold chain where products are transferred from one cold area to another. In a survey conducted in France [1], in which refrigerated products were monitored throughout the cold chain, it was revealed that maintaining appropriate temperature is especially critical in the last three steps in the cold chain (the display cabinet in store, domestic transport and the household refrigerator). Furthermore, several studies reveal consumers' insufficient knowledge about the importance of maintaining the cold chain and carelessness in handling perishable foodstuffs [2,3,4].

Lack of time is the reason consumers frequently and regularly buy chilled and frozen food that either has a short preparation time or does not even require any further heat treatment. Jackson et al. [5] report that chilled and frozen foods including products that can be consumed without further heat treatment represent more than 60 % of the typical shopping basket of an average European consumer. To reduce the risk of temperature mishandling in case of perishable food items, transport in an insulating bag or box is generally recommended. In addition to a high resistance to the transfer of heat, a good insulating material must have various characteristics (depending upon the application); low cost, low moisture susceptibility, ease transportation, consumer appeal, and mechanical strength are the most relevant ones [6]. Furthermore, a clean insulating bag interior is essential to avoid contamination or cross-contamination of food and to prevent changes in sensory properties, especially the adsorption of foreign smells.

In addition to a high resistance to the transfer of heat, a good insulating material must have various characteristics (depending upon the application); low cost, low moisture susceptibility, ease transportation, consumer appeal, and mechanical strength are the most relevant ones.

Evans [7] investigated the effect of the time period, and the manner of transport on a food temperature purchased from a large retail store and placed in a pre-cooled insulating box or left in the boot of a car unprotected. In some products, temperatures in the boot rose up to 40°C during a one-hour car journey during which most of the samples placed in the insulating box did not change their temperature during the transport. Those transported in a boot of a car then required approximately five hours after being placed in a domestic refrigerator before the temperature was again reduced below 7 °C.

A study among Slovenian population revealed that the average time a consumer needs to travel from store to home is 25 min [4], which is less than reported by Derens [1] for French consumers, where the duration of

the domestic transport between retail and the consumer's home is typically an hour. Others report up to or even more than 90 min for 7 % of consumers investigated [8]. The study of Jevšnik et al. [4] also revealed that 51.7 % of the respondents never even thought of using an insulating bag, while additional 33 % believed that an insulating bag is not necessary. Among all respondents (N = 985), only 15.5 % had ever taken an insulating bag to the store when buying perishable foodstuffs, while this percentage was significantly higher among respondents who also believed that the consumer is also responsible for food safety.

In spite of the issue addressed above, there is little evidence regarding the efficiency of insulating boxes; such research is extremely rare and mostly in the context of material testing [6,9]. The aim of the current research is therefore to evaluate the efficiency of insulating bags meant for maintaining the cold chain by consumers during the transport of perishable food items from the store to the their home. The efficiency of insulating bags will be evaluated at different internal and external conditions, also considering their price, with different insulating bags at different prices are available to the consumer.

The aim of the current research is therefore to evaluate the efficiency of insulating bags meant for maintaining the cold chain by consumers during the transport of perishable food items from the store to the their home.

METHODS

Apparatus and materials

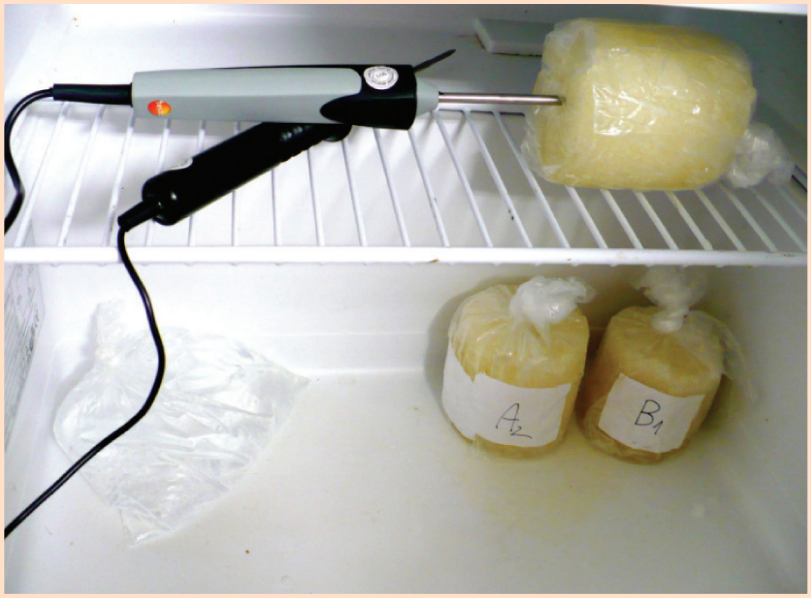
The efficiency of insulating bags available at different purchase prices and one typical bag was tested. All three insulating bags are made of metallized low-density polyethylene (LDPE) with snap-in type closures (polyethylene terephthalate (PET)) and same outside dimensions (approx. 50 cm × 50 cm). A typical bag is made of Polyvinyl chloride (PVC). For further presentation of the data, they were numbered as follows:

- Bag 1: typical PVC bag
- Bag 2: low price insulating bag (€0.79)
- Bag 3: middle price insulating bag (€1.49)
- Bag 4: high price insulating bag (€2.24)

The internal temperature was measured with an artificial "test object" with weight of 246 g. The material used for its preparation is tylose gel (77 % water, 23 % methylcellulose powder). Tylose gel is often used for studying heat transfer during freezing and thawing operations, while its thermal properties are similar to lean beef; it has been previously validated [10]; it is homogeneous and can be reused for several repetitions.

During the measurements of internal temperature, a probe was placed in the interior of the test object. For the collection of data during temperature measurements, a Testo 177-T4 data logger with a -200 °C to +400 °C measurement range, 0.1 °C resolution and ± 0.3 °C accuracy was used. The internal temperature was monitored in five-minute intervals. Measurements were done at two different external air temperatures (15 °C and 30 °C) simulating different outside temperatures. Internal conditions were related to the load of insulating bag during the experiment. Three different situations were tested with *i)* one test object only, *ii)* three

Picture 1:
Test product together with the thermometer probe in the insulating bag



Picture 2:
Test product together with the data logger and thermometer probe in the insulating bag

Picture 3:
Insulating bag with content in the incubator

test objects and *iii)* three test objects plus 1.5 L of water in PET bottle. In all cases, a thermometer probe was inserted in only one test object to monitor internal temperatures. For controlled experimental conditions, a Kambič I-45 CK air temperature incubator (with a volume of 44 L, forced air circulation, 0.1 °C resolution of temperature setting) and a Zanussi ZRG309W refrigerator (with volume of 91 L) were used. The average air temperature in the refrigerator during the measurements was 4.8 °C (SD = 1.0). The average air temperature in the incubator during the measurements was 15.4 (SD = 0.5) and 29.7 (SD = 0.6) respectively.

Experimental procedure

Initially, the test object (together with the thermometer probe) was placed in the refrigerator for 24h to adapt (Picture 1). During each measurement, the internal temperature of the test object was monitored after the first 15 minutes while still in the refrigerator. After 15 minutes, the test object, together with thermometer probe and data logger, was placed into the selected bag (Picture 2). The closed bag was transferred into the incubator for 60 minutes (Picture 3). Afterwards, the test object is again placed in the refrigerator, where the internal temperature was still monitored for additional 300 minutes. The next measurement was executed at least after 15 hours rest of the test object (together with the thermometer probe) in a refrigerator.

RESULTS AND DISCUSSION

The results presented in Table 1 clearly show that insulating bags are not effective in preserving appropriate temperature environment for one hour in the described experimental conditions to which test object was exposed. An evaluation of results in Tab. 1 demonstrates the impact of bag external conditions (temperature outside the bag) as well as bag internal conditions (degree of load) on the test object's internal temperature. Although 30 °C was chosen as an experimental condition, it must be mentioned that in real situations when food is transported in cars, air temperature varies in accordance with solar radiation and cloud cover. Kim et al. [11] report that the temperature difference between the car trunk and outdoor can be up to 15.8 °C with no cloud cover and the highest solar radiation (21.1 MJ/m^2) or just 4.8 °C under the low solar radiation (14.6 MJ/m^2) and maximum cloud cover.

The difference between initial internal temperature of the test object and the intermediate temperature after 60 minutes of exposure to described bag internal and external conditions is expressed as ΔT. An average ΔT comparing only insulating bags (Bags 2–4) was 2.4 °C and 6.2 °C at outside temperatures 15 °C and 30 °C, respectively. An average ΔT of a typical PVC bag (Bag 1) was 2.6 °C and 8.2 °C at outside temperatures of 15 °C and 30 °C respectively. Closer examination shows that ΔT is decreasing in relation to the higher bag number and higher degree of load. Differences between values of ΔT (when comparing different bags) are becoming smaller when the degree of load is increased. Bag 1, used as a control bag, proved to be at least efficient to preserve the initial

Table 1: Internal temperatures of test object at different internal and external bag conditions

EC	IC	Bag nr.	Initial T (°C)	Intermediate T (°C)	Maximum T (°C)	Final T (°C)	ΔT (°C)	Case number
15°C	1	1	4.8	7.9	8.8	5.8	3.1	1
		2	4.8	7.8	8.4	5.4	3.0	2
		3	4.7	7.5	8.1	5.5	2.8	3
		4	5.1	7.4	7.9	5.4	2.3	4
	3	1	4.7	7.1	7.8	5.3	2.4	5
		2	4.5	6.9	7.3	5.1	2.4	6
		3	4.6	6.9	7.3	5.2	2.3	7
		4	4.8	7.1	7.5	5.2	2.3	8
	3+	1	4.9	7.2	7.8	5.5	2.3	9
		2	4.9	7.1	7.4	4.8	2.2	10
		3	4.4	6.4	6.4	5.4	2.0	11
		4	4.8	6.8	7.3	5.5	2.0	12
30°C	1	1	4.8	13.5	15.1	6.7	8.7	13
		2	4.9	12.2	13.6	6.4	7.3	14
		3	5.0	12.3	13.5	5.8	7.3	15
		4	4.7	11.9	13.3	6.4	7.2	16
	3	1	4.6	12.5	13.4	6.1	7.9	17
		2	4.8	11.5	13.0	6.1	6.7	18
		3	4.4	10.8	12.2	5.8	6.4	19
		4	4.7	10.8	12.2	5.9	6.1	20
	3+	1	4.9	12.8	13.2	6.5	7.9	21
		2	4.9	10.5	11.3	6.3	5.6	22
		3	5.1	9.9	11.2	5.3	4.8	23
		4	5.2	9.9	10.6	5.5	4.7	24

Legend: *EC* – Bag external conditions; *IC* – Bag internal conditions; *Initial T* – Initial internal temperature of test object in the refrigerator; *Intermediate T* – Intermediate internal temperature of test object after 1h in selected bag exposed to experimental conditions; *Maximum T* – Maximum internal temperature of test object measured; *Final T* – Final internal temperature of test object after taken out of the selected bag and stored in refrigerator for 5 h; *ΔT* – Internal temperature difference of the test object during one hour exposure to experimental conditions; *1* – one test object; *3* – three test objects; *3+* – three test objects with 1.5L of water in PET bottle.

temperature of the test object. In contrast, the most expensive insulating bag (Bag 4) was the most efficient in comparison to the other bags. Consequently, the smallest *ΔT* is observed for case number 12 and the highest for case number 13 (Tab. 1). Comparing the typical plastic bag with insulating bags also demonstrates that differences of *ΔT* are more obvious at higher outside temperatures (30 °C) and higher degrees of load. Further comparing *ΔT* only between insulating bags (Bags 2–4) reveals that the differences are minimal with no significant impact of their purchase prices.

Closer examination of the maximum internal temperature reached also reveals that when the outside temperature is 30 °C the test object's internal temperature reaches 13.6 °C if stored in insulating bag. Although some previous studies [7] report that during a one-hour car journey most of the samples placed in the pre-cooled insulating box did not change their temperature during the transport, this was not confirmed with our measurements. This could be due to the fact that insulating bags were not pre-cooled, and the relatively small test object (246 g)

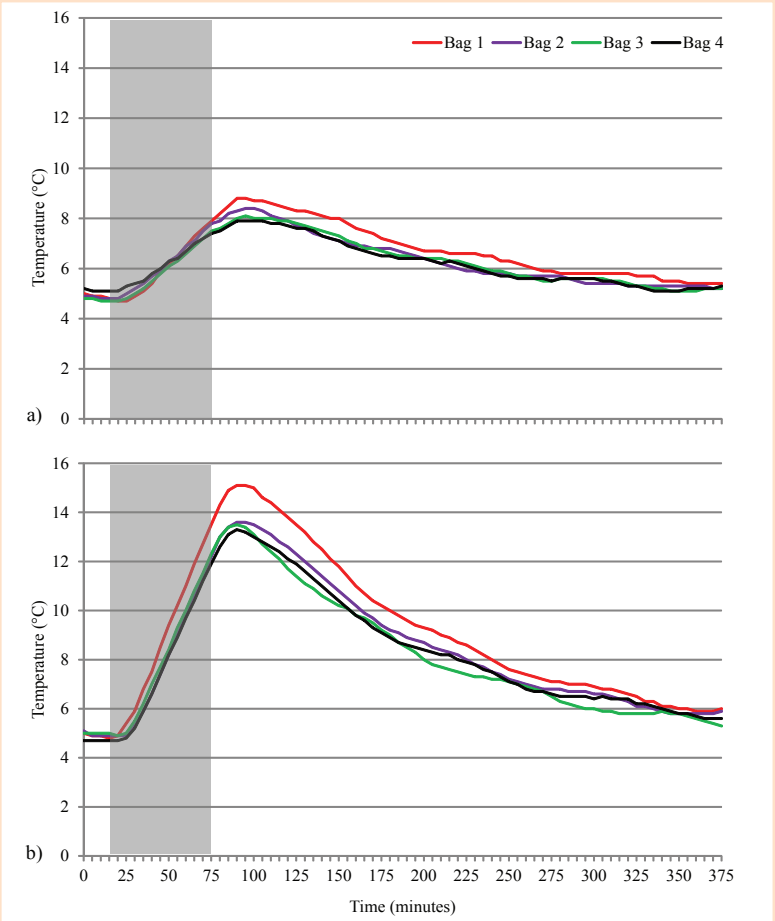


Figure 1:
Comparison of internal temperatures movement of test object placed in different bags at a) 15 °C and b) 30 °C external temperature. The grey area indicates the time period (60 minutes) when the insulated bag and its content were exposed to controlled outside temperature.

used. In our study, bigger test objects were not evaluated. As reported by Kim et al. [11], among the food items examined the temperature dramatically increased immediately after storage in the trunk by food items with the lowest weight.

The maximum internal temperatures (Tab. 1) measured already present a rather favourable temperature environment for the progress of psychotropic microorganisms which grow well at 7 °C and have their optimum at 10–15 °C, depending on nutrient content, pH and the availability of liquid water [12]. However, the higher surface temperature of the test object (not measured) and time of exposure to these temperatures should not be neglected.

Closer examination of the test object's internal temperature rise if placed in different bags at different external temperatures (Fig. 1) reveals that internal temperatures also rise after the test object is placed back into the refrigerator. The maximum internal temperatures of the test object were reached 15 to 20 minutes after placement in the refrigerator, and retained at a maximum level for an additional 10 minutes, exceptionally 15 minutes in cases 6 and 11 (Tab. 1), before they began to drop. Closer examination (Fig. 1) of the test objects' internal temperature inclines between initial and maximum value, calculating the slope, additionally reveals that at both (15 °C and 30 °C) outside temperatures, the internal temperature of the test objects placed in Bag 1 increases faster (higher slope) compared to the insulating bags after exposure to experimental

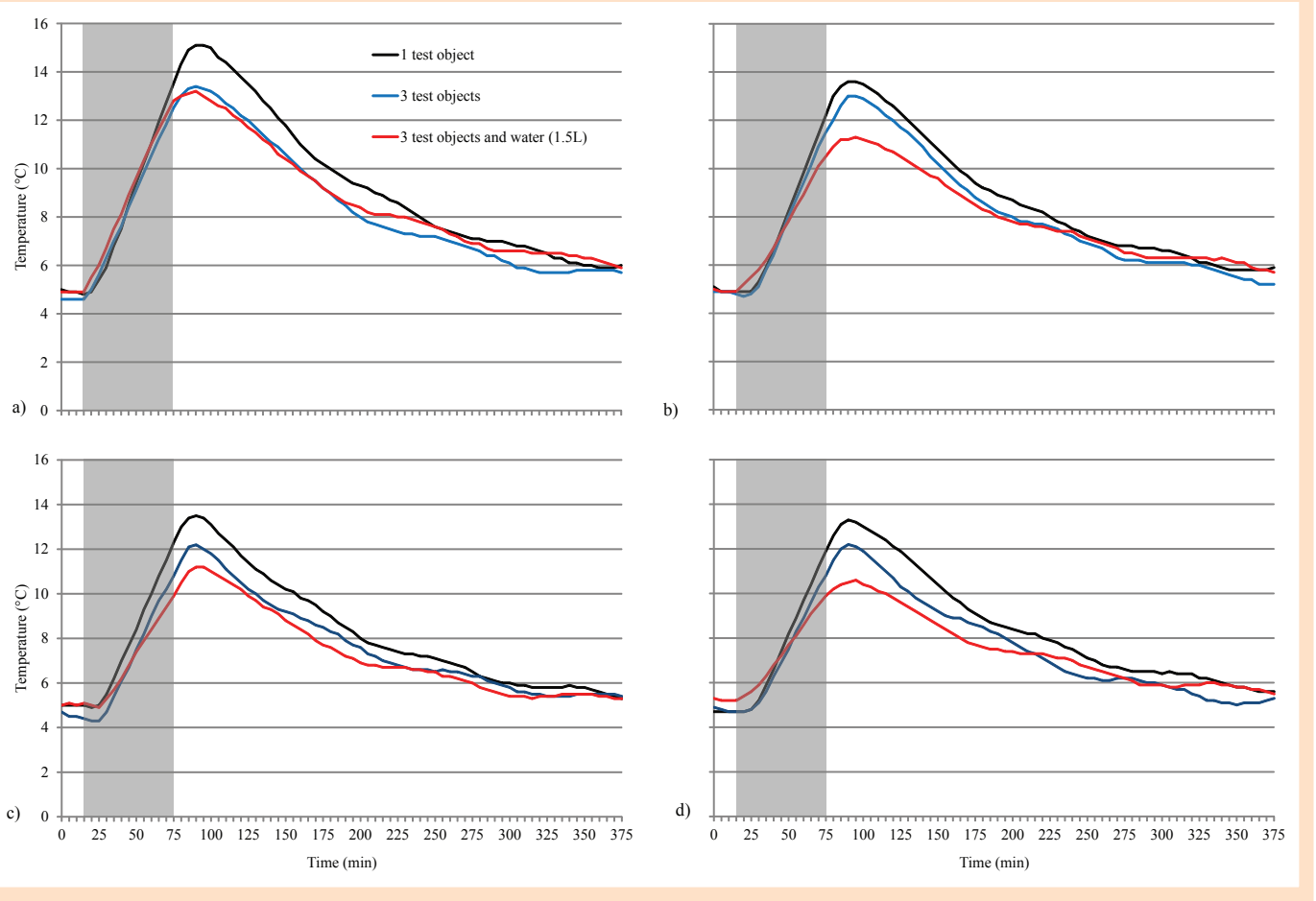


Figure 2:
Comparison of internal temperature movement of test object at 30°C external temperature and different internal conditions in a) bag 1, b) bag 2, c) bag 3 and d) bag 4. The grey area indicates the time period (60 minutes) when the insulated bag and its content were exposed to controlled outside temperature.

conditions. Furthermore, a slope comparison between insulating bags reveals that the value decreases in correlation with the bag's price (Fig. 2). However, this is true only if one test object is present in the bag.

A comparison of the test objects' internal temperature at 30 °C external temperature and different internal conditions demonstrates that increases of internal temperatures of the test object is diminished when the degree of load is increased in all bags examined (Fig. 2a–d). The highest internal temperatures of test objects' are achieved with one test object only. A closer examination of temperature rises in correlation to time also reveals that approximately five hours are necessary after test object is placed in a refrigerator, for the internal temperature to be again reduced below 6 °C.

In this context, consumer behaviour should be taken into account. Godwin & Coppings [13] report that consumers using insulating bags consequently extend the time of transport to their home, which also otherwise differs between different countries [1, 3, 4, 7, 14]. If insulating bags are not as efficient as consumers would expect or believe, irrespective of the outside temperature and degree of load in the bag, the risk for infection or spoilage is increased. Additionally it must be considered that consumers often do not pay any particular attention to the temperatures in domestic refrigerator. As reported by James and others [15], it is clear that many refrigerators throughout the world are already running at higher temperatures than recommended.

From the total cold chain point of view based on the measurement done by Darens et al [1], a refrigerated product spends two thirds of its life in an environment managed by professionals and the rest managed by the consumer. However, professionals in food stores do not always maintain appropriate temperatures. As reported by others [16,17], the temperatures measured differed from the required ones by for up to 10 °C.

Guidelines of good hygienic practices and the principles of the HACCP system in stores [18] recommend to consumers that food items requiring maintaining of the cold chain should be stored in insulating bags during the transport. A commercial insulating bag is a type of shipping container in the form of a bag made of materials with thermal insulation properties used to maintain the temperature of its contents. Most insulating materials utilize low thermal conductivity as a means of restricting the transfer of heat, although radiation and convection are also significant means. Resistance to heat transfer depends on various characteristics that determine the insulating ability of a container [6,19]. The wall thickness affects heat transfer via conduction, the number of surfaces via convection and the number of reflective surfaces (such as aluminium foil) via radiation. According to the manufacturer, the insulating bags used in this study should be effective up to one hour, and are intended for repeated use. However, it has to be stressed that, according to the manufacturers' statement written on the exterior of the insulating bag, they are effective up to one hour for deeply frozen food items.

Guidelines of good hygienic practices and the principles of the HACCP system in stores recommend to consumers that food items requiring maintaining of the cold chain should be stored in insulating bags during the transport.

CONCLUSIONS

The results revealed in this study indicate that insulating bags whose primary purpose is to preserve appropriate temperature environment for deeply frozen food items are not sufficiently effective to preserve appropriate temperature environment for chilled food items. Although insulating bags proved to be more efficient in comparison to the typical PVC bag, the difference was not as significant as expected. Furthermore, the differences between insulating bags are not correlated with their purchase price. The measurements revealed that the internal temperature of the test object varies in accordance with air temperature outside the bag and the degree of load in the bag.

The measurements suggest that insulating bags are not sufficiently effective to preserve chilled foods, especially when not filled with many food products. This suggests a need to modify the insulating bags regarding their effectiveness for chilled foods and highlights the importance of short transport times from the store to home.

Transport between retail and the consumer's home is quite short in comparison to other links in the cold chain, sometimes leading to the idea that the impact of this link on food safety should be less important. Nevertheless, its impact on the quality and safety of the product should not be considered negligible. To obtain exact prove of food safety, microbiological predictive models or microbiological analysis should be performed in the future, establishing an integrated approach of the evaluation of chilled product's safety.

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Original scientific article

Potential applications of rapid microbiological methods for detection of antibiotic residues in wastewater, surface and well water

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ABSTRACT

The goal of the present study was to determine whether the commercial microbiological and tracer assays for the detection of antibiotics in the food were also useful and sensitive enough for testing water samples. Diffusion tests Delvotest® SP-NT and BRT-AiM showed the similar sensitivity to tested antibiotics in spiked water samples. Both tests showed the similar sensitivity to examined antibiotics in water as it was published in milk, while tracer assay BetaStar showed slightly higher minimum detection levels for penicillin and ampicillin but not for cloxacillin. The previous concentration of the samples by lyophilization took place to detect concentrations of antibiotics 100-fold lower than there were the minimum detection limits of the assays. The presence of inhibitory substances in surface and well samples was detected in 16 (16.3 %) cases out of 98 with both ampoule diffusion methods. The positive results were obtained at 15.0 % of surface water samples, while in well water the residues were found also in 16.9 % and 13.6 % samples, using Delvotest SP-NT and BRT-AiM, respectively. The β-lactams were detected with BetaStar in 7.5 % of surface water samples. The 12 wastewater samples from hospitals were contaminated with inhibitory substances in 45.5 % (Delvotest SP-NT) or in 36.4 % (BRT-AiM).

Key words: Antibiotics, microbiological methods, water, screening, contamination

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INTRODUCTION

Antibiotics are pharmaceuticals which are used widely and in large amounts in human and veterinary medicine.

In veterinary practice, they are utilized at therapeutic levels primarily to treat diseases [1]. Cabello [2] reported about the widespread use of prophylactic antibiotics in aquaculture to forestall bacterial infections.

Residues of human and veterinary pharmaceuticals, including a lot of non-biodegradable antibiotics, are introduced into the environment via a number of pathways, primarily from discharges of wastewater treatment plants from hospitals and pharmaceutical industry or land application of sewage sludge and animal manure. They have been consequently widely detected in various environmental matrices including surface water, groundwater, soils, and sediments [3]. Although wastewater treatment plants remove some pharmaceuticals during the cleaning process [2, 4] the removal efficiencies vary from plant to plant. In certain circumstances they even inhibit the working microorganisms in biological wastewater treatement plants [5, 6]. Some antibiotics seem to persist in the environment long time and cause adverse health effects in both humans and wild life [5, 7, 8, 9]. They may lead to the development of antibiotic-resistant strains of microorganisms [9, 10].

Some antibiotics seem to persist in the environment long time and cause adverse health effects in both humans and wild life. They may lead to the development of antibiotic-resistant strains of microorganisms.

Residues may enter the food chain and are found at different concentration levels not only in drinking water, but also in products of animal origin, such as milk, eggs and meat and can cause human health problems (e.g. the induction of allergic reactions in hypersensitive individuals). The prevention of antibiotic residues in milk and meat is crucial in order to avoid losses in fermentation processes using microorganisms as starter cultures [1].

Antibiotic residues in edible animal products are of great concern to regulatory agencies and consumers, so reliable screening methods for rapid, selective and sensitive detection of these residues were developed to ensure food safety [11]. In general, analytical methods for monitoring antibiotic residues in food can be classified in three groups:

Biological methods based on bacterial growth inhibition. They are not selective and can cover several chemical classes of active analytes but do not allow the identification of individual analytes.

The toxic or genotoxic effect of different substances, including antibiotics can be detected by bioassays, using bacteria *Vibrio fisheri*, *Microcystis aeruginosa* (cyanobacteria), *Brachionus calyciflorus* (rotifer), *Thamnocephalus platyurus* (crustacean anostraca), *Daphnia magna* (crustacean cladocera), *Danio rerio* (teleostei), *Pseudokirchneriella subcapitata* (green algae), and some others [12, 13].

The presence and concentrations of specific antibiotics in water samples are determined by more sensitive physicochemical methods, like solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) [14, 15, 16].

Physicochemical methods (e.g. TLC, GC, LC, HPLC, capilary electrophoresis, LC/MS) distinguish the chemical structure and molecular characteristics of analytes by separation of molecules and the detection of signals related to molecular characteristics. They detect the concentration and type of antibiotics in tested sample. They are time – consuming, expensive and require complex laboratory equipement and trained personnel [11, 17].

Biochemical or tracer methods**,** like ELISA, RIA, etc., detect molecular interactions between analytes and antibodies or receptor proteins. They are either selective for a family of analytes having related molecular structures or are sometimes analyte specific [11].

The goal of the present study was to determine whether the methods for the detection of antibiotics in food were also useful and enough sensitive for testing water samples. We focused on analytical methods on commercial kit tests that allow fast, sensitive detection of antibiotic residues with minimum sample treatment. Once these procedures were optimized, they were applied to the analysis of water samples collected from some major Slovenian streams, groundwater from wells and wastewaters.

The goal of the present study was to determine whether the methods for the detection of antibiotics in food were also useful and enough sensitive for testing water samples.

MATERIAL AND METHODS

Environmental water samples

A total of 110 water samples, collected in the period from two seasons: December 2009 to March 2010 and June to September 2010 were tested for the presence of inhibitory substances. Fifty-nine out of 110 samples were groundwater samples from individual wells, 40 were surface water samples (streams, rivers) and 11 samples were wastewater samples from hospitals, clinical departments and one farmaceutical factory (**Table 3**). The sampling sites were selected randomly in rural and urban areas, distributed throughout the country. The temperatures of winter and summer samples were between 4 °C to 13 °C and 10 °C to 21 °C, respectively, the rainfall quantity was measured as well.

The sampling sites were selected randomly in rural and urban areas, distributed throughout the country.

From each of the testing sites 1–2 samples were collected, not all of them were tested in each of the sampling period.

Preparation of environmental samples

One litre of water sample was collected in duplicates into appropriate sterile glass bottle, approximately 20 cm below the surface of the water in two different sides of each stream and transferred to the laboratory at temperatures from 4 to 10 °C in maximal two hours. All samples were filtered through 0.45 μm filters (11306-50-N, SartoriusStedim, Germany) and stored at -20 °C until they were analysed. The pH values in well and surface water samples ranged between 6.5 and 7.3 while in wastewater between 6.8 and 8.5.

Spiked and standard samples

The spiked samples were prepared with defined concentrations of antibiotics. Standard solutions of antibiotics benzyl penicillin, ampicillin, cloxacillin, erythromycin, kanamycin, nalidixic acid and chloramphenicol, were prepared in concentrations, which are minimal detection limits for used methods for milk. To study the matrix effect, we prepared samples of water and milk with the same concentrations of dissolved antibiotics. Each antibiotic was dissolved in sterile distilled water in different concentrations. For the preparation of milk samples, the antibiotics were first dissolved in distilled water as stock solutions and then in reconstituted skim milk (Skim milk powder, 1.15363, Merck, Germany) as well. The proportion of the added aqueous standard solution in the final milk dilution step was less than 1 %. The selection of concentrations for the determination of the senstivity for each test was based on the detection limits mentioned by producers, together with at least one concentration step higher and two concentration steps lower [18] (**Table 1**). The spiked samples were divided into three subsamples and frozen at -20 °C ± 2 °C. The test kits with different batches were used for detecting the antibiotics in each subsample.

The selection of concentrations for the determination of the senstivity for each test was based on the detection limits mentioned by producers, together with at least one concentration step higher and two concentration steps lower.

We 100-folded the concentration of water samples and thereby detected lower concentrations of antibiotics with the same methods. For this purpose 300 ml of the sample was lyophilizated using Freeze Dryer Alpha 2-4 LSC, Christ (Germany). For each concentration of antibiotic there were prepared two parallel samples. After lyophilization one of them was dissolved in 3 ml of sterile nutrient broth [19], and the other in sterile distilled water, with intention to investigate the potential impact of the diluent on the sensitivity of the methods. All working solutions were prepared freshly at the same day of analysis. According to the recommendations of the International Dairy Federation the pH of the sample should be higher than 6 [20]. The pH values of resuspended concentrates prepared after lyophilization were between 6.8 and 8.2.

Standard samples Penicillin G Standard, full cream milk lyophilizate 4 ng/mL (9143, AiM GmbH, Germany), and Inhibitor Free Milk, full cream milk lyophilizate, (9150, AiM GmbH, Germany) were used as positive and negative control. The nutrient broth (Merck, Germany) and distilled water were used as negative control too. The antibiotic discs with gentamycin, GM 10 μg, penicillin G, P 10 IU and streptomycin, S 10 μg (Becton Dickinson, Great Britain) were used as standards for detection of the sensitivity at disc diffusion methods.

METHODS

For detection of inhibitors and medical residues in water, there were established microbiological ampoule diffusion methods BRT-AiM (tubes for single sample, 3040, Neogen Corporation, USA) and Delvotest® SP-NT (DSM Food Specialities, The Netherlands), disc diffusion methods with test bacteria *Geobacillus stearothermophilus* var.

Table 1:
The sensitivity of the methods used in the experiment and concentrations of tested antibiotics in spiked milk and water samples.

Antibiotics	Concentration of antibiotic in the sample µg/L	Concentration of antibiotic in the water after lyophilisation µg/L[a]	Delvotest SP-NT		BRT		Discs *G. s.*[b] inh. zone (mm)[c]		Discs *B. s.*[d] inh. zone (mm)[c]		Beta Star	
			Milk	Water	Milk	Water	Milk	Water	Milk	Water	Milk	Water
β-lactams												
Benzyl-Penicillin G potassium salt (13750, Sigma Aldrich, USA)	1.0		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	1.5		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	2.5[f]		+[f]	+	+	+	6±0	6±0	6±0	6±0	-	-
	4.0		+	+	+	+	**8±0.1**	6±0.2	6±0	6±0	+	+
	25		+	+	+	+		**37.3±0.4**	**19.2±0.2**	**18.2±0.2**	+	+
	250		+	+	+	+		40.3±0.2	NM[e]	NM[e]	+	+
	0.001	1.0		-		-		6±0		6±0		-
	0.015	1.5		-		-		6±0		6±0		-
	0.025	**2.5**		+		+		6±0		6±0		-
	0.04	4.0		+		+		6±0.7		6±0		-
	0.06	**6.0**		+		+		**65.8±1.2**		**19±0**		+
	0.24	24		+		+		48.7±2.4		18.8±0.9		+
	0.25	25		+		+		44.2±0.2		18.8±0.8		+
	2.5	250		+		+		15.8±0.5		NM[e]		+
Ampicillin sodium salt (A9518, Sigma Aldrich, USA)	1.0		-	-	-	-		6±0			-	-
	1.5		-	-	-	-		6±0			-	-
	2.5		+	+	+	+		6±0			+	-
	4.0		+	+	+	+		6±0			+	+
	16		+	+	+	+		**6.8±0.2**			+	+
	25		+	+	+	+		34±0			+	+
	250		+	+	+	+		16.5±0.5			+	+
	0.025	**2.50**		+		+		6±0				-
	0.04	4.00		+		+		6±0				-
	0.16	**16**		+		+		**6.2±0.2**				+
	0.25	25		+		+		36.0±0.9				+
	2.5	250		+		+		16.0±0.6				+
Cloxacillin sodium salt monohydrate (C9393, Sigma Aldrich, USA)	**1**		-	-	+	+	6±0	6±0	6±0	6±0	-	-
	4		-	-	+	+	6±0	6±0	6±0	6±0	+	+
	10		-	-	+	+	6±0	6±0	6±0	6±0	+	+
	20		-	-	+	+	6±0	6±0	6±0	6±0	+	+
	40		-	-	+	+	6±0	6±0	6±0	6±0	+	+
	100		+	+	+	+	6±0	6±0	6±0	6±0	+	+
	0.01	**1**		-		+		6±0		6±0		-
	0.04	**4**		-		+		6±0		6±0		+
	0.10	10		-		+		6±0		6±0		+
	0.20	20		-		+		6±0		6±0		+
	0.40	40		-		+		6±0		6±0		+
	1	100		+		+		6±0		6±0		+
	2	200		+		+		6±0		6±0		+

Antibiotics	Concentration of antibiotic in the sample µg/L	Concentration of antibiotic in the water after lyophilisation µg/L[a]	Delvotest SP-NT		BRT		Discs *G. s.*[b] inh. zone (mm)[c]		Discs *B. s.*[d] inh. zone (mm)[c]		Beta Star	
			Milk	Water	Milk	Water	Milk	Water	Milk	Water	Milk	Water
Macrolydes												
Erythromycin (E5389, Sigma Aldrich, USA)	40		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	75		+	-	+	+	6±0	6±0	6±0	6±0	-	-
	150		+	+	+	+	**16±0.2**	**16±0.0**	6±0	6±0	-	-
	300		+	+	+	+	16.6±0.1	16.4±0.7	6±0	6±0	-	-
	0.001	0.1		-		-		6±0		6±0		-
	0.1	10		-		-		6±0		6±0		-
	0.4	**40**		-		+		6±0		6±0		-
	1.5	**150**		+		+		**18±0.6**		6±0		-
Amynoglicosides												
Kanamycin sulfate (K4379, Sigma Aldrich, USA)	37		-	-	-		6±0	6±0	6±0	6±0	-	-
	75		-	-	-		6±0	6±0	6±0	6±0	-	-
	378		+	+	+	+	6±0	6±0	6±0	6±0	-	-
	3785		+	+	+	+	6±0	6±0	6±0	6±0	-	-
	7570		+	+	+	+	6±0	6±0	6±0	6±0	-	-
	37850		+	+	+	+	6±0	6±0	6±0	6±0	-	-
	0.378	37.8		-		-		6±0		6±0		-
	0.757	75.7		-		-		6±0		6±0		-
	3.785	**378.5**		+		-		6±0		6±0		-
	37.85	**3785**		+		+		6±0		6±0		-
	75.70	7570		+		+		6±0		6±0		-
	378.50	37850		+		+		6±0		6±0		-
	3785	378500		+		+		6±0		6±0		-
	37850	3785000		+		+		6±0		6±0		-
Quinolones												
Nalidixic acid sodium salt (N3143, Sigma Aldrich, USA	0.05		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	1		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	5		+	+	+	+	**8±0.1**	6±0	6±0	6±0	-	-
	25		+	+	+	+	9±0	6±0	6±0	6±0	-	-
	0.01	1		-		-		6±0		6±0		-
	0.050	**5**		+		+		**7.7±0.5**		6±0		-
	0.25	25		+		+		10±0.2		6±0		
	25	2500		+		+		6±0		6±0		-
	255	25520		+		+		6±0		6±0		-
Others												
Chloramphenicol (CO378, Sigma Aldrich, USA)	25		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	50		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	250		-	-	-	-	6±0	6±0	6±0	6±0	-	-
	2500		+	+	+	+	6±0	6±0	6±0	6±0	-	-
	5000		+	+	+	+		6±0		6±0		-
	0.025	2.5		-		-		6±0		6±0		-
	0.25	25		-		-		6±0		6±0		-
	2.5	250		-		-		6±0		6±0		-
	25	**2500**		+		+		6±0		6±0		-
	50	5000		+		+		6±0		6±0		-

[a] – after 100-fold concentration with lyophilization followed by resuspension with distilled water or nutrient broth; [b] – *G. s.*: disc diffusion method with *Geobacillus stearothermophilus* var. *calidolactis*; [c] – diameter of inhibition zones in mm (mean values of 3 measures and the average deviations of the mean); [d] – *B. s.*: disc diffusion method with *Bacillus subtilis*; [e] – NM: not measured; [f] – The detection limits of the methods, representing 95 % positive results for each antibiotic in the experiment, were highlighted in the **bolt** script.

calidolactis C953 (ATCC7953, 1.11499, Merck, Germany) which is added to the melted sterile agar medium according to Kundrat (1.10662, Merck, Germany), and *Bacillus subtilis* strain BGA (DSM618, 1.10649, Merck, Germay) in Test Agar pH 7.2 for the inhibitor test (1.15787, Merck, Germany). The tracer method BetaStar (Neogen Corporation, USA) is a receptor binding assay, which detects penicillins and cephalosporins.

The procedures were carried out following manufacturer's instructions and recommendations of previous publications [1, 20, 21, 22, 23, 24, 25, 26, 27, 28].

The spiked samples were tested in triplicates using different assay batches and environmental samples in duplicates as well.

The statistical analyses were calculated by using IBM SPSS Statistics 20 programme. The statistical analysis included analysis of Pearson Chi-Square between samples. Two-sided asymptomatic significance was set at α=0.05.

RESULTS AND DISCUSSION

The surface waters and especially underground water are sources for drinking water supplies, so its physiochemical and microbiological quality is very important.

The spiked samples were tested in triplicates using different assay batches and environmental samples in duplicates as well.

Most classical bioassays for detecting genotoxic substances generally in water samples have not proven very sensitive to antibiotics or are not fast enough screening tools [12, 13], their minimal detection concentrations for antibiotics are higher than those that have proven at routine methods for the detection of antibiotics in food.

We assessed the suitability of some commercial microbiological and tracer methods routinely used in food control for detection of antibiotics in water. Their minimal detection levels for single antibiotic residues are mostly in the concentrations prescribed as MRL in food samples [29] (**Table 2**).

The concentrations of antibiotics residues are in water sources according published reports lower than MRLs for food. The concentrations of antibiotics in streams were up to 0.694 μg/L [30]; up to 1.435 μg/L [16] up to 2.3 μg/L [31, 32], or even up to 6.72 μg/L [33], depending on the type of detected antibiotic, the sample, the area and the season of sampling.

The highest concentrations of quinolones in surface water were from 0.3 to 1.3 μg/L, while the mean values of β-lactams were found around 0.25 μg/Land amynoglycosides 0.04 μg/L [9, 31]. Feitosa-Felizzola and Chiron [33] reported about the concentrations of clarithromycin and oxitetracycline in streams 0.02 and 0.08 μg/L, respectively (**Table 1**).

The maximal concentrations of antibiotics in wastewater samples from hospitals were in the range from 0.01 to 15 μg/L [31], from 11 to

69.570 ng/L [32] or from 0.0039 μg/L to approximately 27 μg/L [34]. Brown [35] and Kümmerer [36] detected β-lactams in hospital wastewater in ranges even from 0.85-80 μg/L.

These values are in most cases, particularly in waste waters, approximately 100-fold lower than the MRLs and minimal detection concentrations obtained by routine methods used in food industry. In order to use these routine microbiological methods for detection of antibiotics on the levels found in water, samples should be concentrated in this way, that we could still observe a wide range of different groups of antibiotics. Many antibiotics are sensitive to some solvents or high temperatures, so the chosen procedures of samples preparation should not change their concentration or activity. In our experiment we used the lyophilization of the samples, which is recommended for preparing of test samples for validation of microbial inhibitor tests for ISO 13969/IDF 183 [18]. This procedure would not affect the sensitivity of the method, the activity of the test bacteria, larger changing in pH, persistence of wider range of antibiotics which can be present, and composition of water samples. Hirsch [4] used this technique for preconcentration the water samples before quantification the antibiotics using HPLC-electrospray-tandem-mass spectrometry. Some other ways of concentration, like evaporation and thermization could lead the degradation of antibiotics [37].

The sensitivity of the assays for detecting antibiotics in spiked water samples

The chosen methods and concentrations of tested antibiotics as well as minimum detection limits using the standard solutions of antibiotics are represented in **Table 1**. With Delvotest SP-NT we detected penicillin and ampicillin in concentrations 2.5 μg/L of water sample. After 100-fold concentration of the samples using lyophilization this minimal detection sensitivity was 0.025 μg/L. The minimal concentrations of cloxacillin, erythromycin, kanamycin, nalidixic acid and chloramphenicol, where we obtained the positive reaction of Delvotest, were at least 100 μg/L, 150 μg/L, 378500 μg/L, 5 μg/L and 2500 μg/L of sample, respectively. These values were after concentration decresed 100-fold for each antibiotic (**Table 1**).

The detection levels of β-lactams penicillin and ampicillin were in spiked water samples the same as Mitchell [38] obtained for milk. Delvotest was slightly less sensitive to cloxacillin and chloramphenicol, and more sensitive to erythromycin as it was reported for milk samples [24, 38].

The sensitivities of BRT-AiM towards penicillin, ampicillin, cloxacillin, erythromycin, kanamycin, nalidixic acid and cloramphenicol were in concentrations of at least 2.5 μg/L, 2.5 μg/L, 1 μg/L, 75 μg/L, 378500 μg/L, 5 μg/L and 2500 μg/L of sample, and after lyophilisation 0.025 μg/L, 0.025 μg/L, 0.01 μg/L, 0.75 μg/L, 3785 μg/L, 0.05 μg/L and 25 μg/L for each antibiotic, respectively. Our results showed the lower detection limit for cloxacillin, than it is reported for BRT-AiM test for milk [39, 40] (**Table 2**).

Table 2:
Limits of detection of tested methods towards antibiotics (μg/L) used in the experiment and MRLs for cattle milk.

Drugs	Delvotest[a]	BRT[b]	Disc *G. s.*[c]	Disc *B. s.*[c]	BetaStar[d]	MRL[e]
Penicillines						
Benzylpenicillin	1-2	2-3	6	18	2-4.8	4
Ampicillin	4	2-3	5	-[g]	4-7	4
Cloxacillin	20	20-30	35	-	6-9	30
Macrolides						-
Erythromycin	40-80	40-60	225-600	100		40[f]
Others						-
Chloramphenicol	-	-	-	10000	-	-
Aminoglycosides						
Kanamycin	-	-	28000	-		150[f]
Quinolones						
Nalidictic acid	-	-	-	-	-	-

[a][24, 41]; [b][39]; [c][20];[d][28]; [e][44, 48]; [f][49]; [g] not mentioned

BRT-AiM test and Delvotest showed very similar sensitivity to spiked antibiotic concentrations in water samples, except BRT-AiM test was according our results slightly more sensitive to cloxacillin and erythromycin. *G. stearothermophilus* var. *calidolactis* is the test organism used in both assays which have consequently simmilar sensitivity. They differ among themselves only in the fact, that the color indicator at Delvotest SP-NT reacts to changes in pH values, while at the BRT-AiM test is sensitive to changes in redox potential. The minimal detection limits could be in some cases even lower and more precise if we have used a larger number of spiked samples with minnor differences in the concentrations of the antibiotics.

The satisfactory sensitivity of these two diffusion methods towards amynoglicoside kanamycin and even nalidixic acid as representative of quinolons is delightful, particularly we did not find any limits for these two antibiotics in milk.

Both assays are sensitive not only to a wide range of β-lactams but also to representatives of macrolides, amynoglycosides, lincosamides, sulphonamides etc. as well [24, 39, 40]. It is important, that they can be applicable for screening of samples with a wide range of pH values higher than 5.5 [41].

Some adaptations of the Delvotest and BRT-AiM protocols were required to produce results from environmental samples. Smith [19] recommended that the water samples should be transferred into a nutrient media to stimulate the bacterial spores to germination and then the vegetative cells to rapid growth and respiration.

We obtained some differences in results between samples, dissolved after lyophilization in water and in broth. The samples with 37.8 μg/L of kanamycin and 255 μg/L of nalidixic acid, dissolved in nutrient broth showed with BRT-AiM assay positive reaction. On the contrary, the

negative reaction at the broth sample with 0.0504 μg/L of nalidixic acid using Delvotest SP-NT was observed as well. In other spiked samples there were no differences in results between samples resuspended in nutrient broth and water.

The standard control samples with deffined concentrations of penicillin were used to check the correct procedure of Delvotest SP-NT and BRT-AiM, while the end points of incubation were determined as the time at which the blanks (distilled water, broth) turned yellow. We must point out that we had to extend the incubation for 30 minutes and it took at both assays from 3 hours 30 minutes, regardless of weather it was used nutrient broth or water for resuspendion of lyophilized samples.

BetaStar is sensitive to β-lactam antibiotics penicillin, ampicillin and cloxacillin in milk in concentrations between 2 to 9 μg/L [27, 28]. Our examination of spiked water samples using BetaStar showed slightly higher minimum detection levels for penicillin. The reaction was negative in the test samples with all β-lactams in concentrations of 2.5 μg/L and positive at 6 μg/L, 10 μg/L and 16 μg/L of penicillin, cloxacillin and ampicillin, respectively. In concentrated samples the minimal sensitivity values were 100-fold lower. We also agree with previous reports, that there was observed the equal sensitivity to cloxacillin in the comparasion to reports for milk samples [38, 42] (**Table 1, table 2**). The repeatability of the test was very good and the results were not significantly influenced by small changes (e.g. pH values) in the protocol [28].

Matrix effect was minimal and did not significantly affect on the results.

Calculation of the Chi-Square statistical tests indicate that there were statistically significant relationships between the results obtained by Delvotest SP-NT, BRT-AiM test and BetaStar ($p<0.05$). A comparisson of all three methods shows high correlation ($p<0.05$) and therefore relevance of tested methods. We also found statistically significant relationships between the results of the determination of the antibiotics in milk and water samples and in samples before and after concentration as well ($p<0.01$). Matrix effect was minimal and did not significantly affect on the results (**Table 3**).

More than 6.0 μg of penicillin per litre of water or broth was detected also with both disc diffusion methods. The inhibition zone around disc with 25 μg/L of ampicillin and 150 μg/L of erythromycin on the medium seeded with *G. stearothermophilus* var. *calidolactis* was obvious in all three repetitions, while bacteria *B. subtilis* was not inhibited. The inhibition zone was measured also arround the disc with nalidixic acid in concentration 5 μg/L, but not in higher concentrations used (**Table 1**).

The disc diffusion methods were in our experiment less sensitive than ampoule diffusion methods Delvotest and BRT-AiM. The inhibition zones were at both disc diffusion assays against expectations at higher concentrations of antibiotics in spiked samples smaller than at lower concentrations.

Disc diffusion method with *B. subtilis* was sensitive only to penicillin (**Table 1**) in spite of Okerman [43] reported about positive reaction to

cephalosporines, some quinolones, lincosamides, macrolides, aminoglycosides, and sulphonamides as well. Its sensitivity depends on the pH of the medium and the constitution of the sample matrix. The pH values of the agar medium were targeted to 7.2, because this assay is considered to be according producer's instructions under these conditions slightly less sensitive to penicillin, gentamycin and streptomycin, but extra sensitive to sulfonamides [21, 43]. All used methods were especially sensitive to β-lactam antibiotics [44]. These antibiotics still comprise roughly half of the antibiotic market worldwide. Mostly combined with clavulanic acid or other β-lactamase inhibitors are still the most frequently administered drugs in parental and intra-mamary mastitis therapy in veterinary medicine. They have been reported to dominate in human medicine and the overall antibiotic concentration in some sewage influents as well [26, 28].

In spite of these antibiotics tend to be significantly reduced in concentrations during biological process in wastewater treatment plants [31, 34], some of them showed certain anaerobic biodegradation only after 60 days [5]. Furthermore, they were sporadically reported in effluent, which may indicate that although their pseudopersistance may be occuring due to their continual discharge [31]. Huang [34] identify that antibiotics of sulphonamides and fluoroquinolones are the most likely water contaminants, followed by macrolides. These groups were still detected in wastewater treatment plants effluents, because the average removal rate of greater than 80 % for all of them [31] The representatives of these two groups of antibiotics were well detected with the methods chosen in our experiment.

Huang identify that antibiotics of sulphonamides and fluoroquinolones are the most likely water contaminants, followed by macrolides.

Antibiotic residues in well water, streams and wastewater samples

The data about the presence of antibiotics in Slovenian ground water, drinking water surface water and wastewater have not been published yet. The presence of inhibitory substances was detected by Delvotest SP-NT in 16 (16.3 %) and BRT-AiM assay in 14 (14.3 %) out of 99 surface and well samples. The positive results were obtained at 15.0 % of surface water samples, while in well water the residues were found also in 16.9 % and 13.6 % samples, using Delvotest SP-NT and BRT-AiM, respectively. The antibiotics from β-lactam group were detected with BetaStar in 7.6 % of surface water samples. As it was expected, the wastewater samples were contaminated with inhibitory substances in even 45.5 % (Delvotest SP-NT) or in 36.4 % (BRT-AiM). The β-lactams were determined in 18.1 % of them (**Table 4**). Using discs diffusion methods we did not get positive results, except at one wastewater sample. Generally there were no obvious differences in sensitivity between BRT-AiM test and Delvotest SP-NT. In three cases (2.7 %) out of 110 samples gave Delvotest SP-NT positive and BRT-AiM negative result.

The presence of antibiotics in larger number of water samples from individual wells is a major concern. In rural areas, water from domestic

Table 3:
Statistically significant relationships between Delvotest SP-NT, BRT-AiM test and BetaStar and between types of samples (Pearson Chi-Square with one degree of freedom).

Methods/samples	Chi-Square Value	2-sided asymptomatic significance (p)	R^2
Analysis of methods comparison			
Delvotest SP-NT : BRT	56.821 (min 15.52)[d]	<0.001	0.494
Delvotest SP-NT : BetaStar	7.453 (min 9.05)[d]	0.006	0.128
BRT : BetaStar	21,290 (min 3.62)[d]	<0.001	0.367
Analysis of matrix effect			
Delvotest SP-NT (M)[a] : Delvotest SP-NT (V)[b]	33.197 (min 2.21)[d]	<0.001	0.897
BRT (M) : BRT (V)	37.000 (min 3.89)[d]	<0.001	1.000
BetaStar (M) : BetaStar (V)	15.033 (min 2.21)[d]	<0.001	0.790
Analysis of concentration effect			
Delvotest (V) : Delvotest (Conc)[c]	30.00 (min 5.63)[d]	<0.001	1.000
BRT (V) : BRT (Conc)	21.232 (min 2.70)[d]	<0.001	0.707
BetaStar (V) : BetaStar (Conc)	7.350 (min 1.67)[d]	0.007	0.490

[a](M): milk sample; [b](V): water sample; [c](Conc): sample after concentration using lyophilisation; [d] The minimum expected count.

Table 4:
The presence of inhibitory substances in environmental water samples detected with methods used in the experiment.

Samples	Total	Number (%) of positive samples				
		Delvotest[a]	BRT[b]	Disc *G. s.*[c]	Disc *B. s.*[c]	Beta Star[d]
Surface water	40 (36.4)	6 (15.0)	6 (15.0)	0 (0)	0 (0)	3 (7.5)
Well water	59 (53.6)	10 (16.9)	8 (13.6)	0 (0)	0 (0)	0 (0)
Wastewater	11 (10.0)	5 (45.5)	4 (36.4)	1 (9.0)	0 (0)	2 (18.2)
Total	110 (100)	21 (19.1)	18 (16.4)	1 (9.0)	0 (0)	5 (4.5)

[a] Delvotest SP-NT ampoule format, control time: time of negative control colouring yellow [24, 41];
[b] BRT-AiM test [39];
[c] Disc diffusion method with *Geobacillus stearothermophilus* and *Bacillus subtilis* [20, 42];
[d] Tracer assay (Neogen Corporation, USA) [28];

wells, supplied mostly by groundwater, is often used by people for drinking, watering livestock and irrigation of vegetables. Groundwater is a major contributor to flow in many streams and rivers and thus, has a strong influence on river and wetland habitats for plants and animals [45].

In some countries there are no regulations requiring that livestock farms must have a wastewater treatment plants, so that their waste water with undergraded antibiotic residues passed directly through the groundwater and surface water.

Barnes [45] found the veterinary and human antibiotic sulfamethoxazole in 23 % out of 47 groundwater samples, while Arikan [30] detected the same anthibiotic in 19 % of samples in river stations. Chlortetracycline (19 % detection) and oxytetracycline (15 % detection) were the most frequently detected of the TCs group of antibiotics of the river stations in his study.

Watkinson [31] detected the antibiotics at quantifiable concentrations in more than 50 % out of the 81 surface water samples in South-East Queensland, Australia, which was three times more than in our study. Wang [46] (2010) found four fluoroquinolone antibiotics in 77.5 % of tap water samples from Guangzhou and 100 % of samples from Macao water area.

Hirsch [4] reported about presence of sulfonamide residues in four out of 59 ground samples in agricultural areas in Germany.

The larger differences in the presence of inhibitory substances between winter and summer samples were not estimated. We detected them in 15.2 % of winter samples and 18.5 % of summer samples from individual wells. The specimens from surface waters were positive in 7.7 % of cases in winter and in 29.4 % of cases in summer season. Only twice out of 99 samples the antibiotics were detected in both seasons at the same sampling place. On the contrary, Arikan [30] obtained more samples with positive detections for antibiotics from the group tetracyclines and sulfadrugs in agricultural watershed reivers in USA in the December (winter) collections, followed by collections in June and September. Higher levels of clarithromycin in winter season determined also Feitosa-Felizzola and Chiron [33] in river water in Southern France.

The larger differences in the presence of inhibitory substances between winter and summer samples were not estimated.

Tong [47] reported about average concentrations of eight tested antibiotic residues in groundwater and lake water, respectively, 1.6-8.6 and 5.7-11.6 ng/L in summer; respectively, 2.0-7.3 and 6.7-11.7 ng/L in winter.

It is difficult to compare our results with the publications of other authors, because they mainly reported about the presence of individual antibiotics in waters. Their results were observed by using the precision physico-chemical methods. In comparison with the physicochemical methods the microbiological methods used in our experiment are faster, require unexpensive apparatus and smaller amount of samples. Furthemore, they are more sensitive to antibiotics than standard bioassays for detection the toxic or genotoxic substances in water. The residues of antibiotics according to the published data are obviously very common in the waters, sometimes even in drinking water, which is a great concern.

It is difficult to compare our results with the publications of other authors, because they mainly reported about the presence of individual antibiotics in waters.

Maximum concentrations of antibiotics in the water in the international legislation have not been specified yet. So it would be necessary to define the statutory MRLs in waters too. MRLs for most antibiotics in milk are defined. The MRLs in the water should be probably similar or slightly lower, as in the milk. In this circumstances might be some commercial microbiological assays for determing the inhibitory substances including β-lactams and some other most often prescribed antibiotics in veterinary and human medicine, useful and sensitive enough for routine monitoring of water samples. These positive samples can be than confirmed by immunological or/and chemical assays.

CONCLUSIONS

We can assume that particularly Delvotest SP-NT and BRT-AiM test could be at the appropriate preparation of the samples, useful for routine screening detection of β-lactams and some other antibiotic groups in water, especially in waste waters.

Their minimum detection concentrations in water were comparable to those in milk.

The lyophilization of the samples was used to increase the sensitivity of methods.

Inhibitory substances were obtained in 15.0 % of the Slovenian surface water samples.

In well water the residues were found in 16.9 % of the samples.

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Original scientific article

New ballast water treatment system – combination of filtration, hydrocyclone and cavitation tehnologies

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ABSTRACT

Ballast water released from the cargo ships often acts as an inoculation mechanism for a large number of non-indigenous species that can have an important influence on marine ecosystem's change and even devastation.

The IMO's "Convention for the Control and Management of Ships' Ballast Water and Sediments" demands the establishment of ballast water management system which should solve the question of uncontrolled taking and the operations connected to ballast water releasing. Also, it has been planned to complete the transition to ballast water treatment system.

The faculty team work on project which main aim is to examine and develop the principle of technology for ballast water treatment, whose action is based on the use of the combination of filtering technology, hydrocyclone and cavitation.

Until now, the project has proved the possibility of hydrodynamic cavitation appearance inside the hydrocyclone that has been an unexplored phenomenon so far. The next step of the project is to prove hydrodynamic cavitation effectiveness in a joint operation with hydrocyclone which should offer a solution for disabling and removing marine organisms from ballast water.

Key words: Ballast water, convention for the control and management of ships' ballast water and sediments, combination of filtering technology, hydrocyclone and cavitation

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INTRODUCTION

Ballast water from ships is considered the most important vector of non-indigenous organisms in aquatic ecosystems [1]. The transport of the world's ship ballast water stands at about 12 E9 t per year, and it is estimated that the ship's ballast tanks can transfer at least 10 000 different species of organisms [2].

The ballast water discharged from ships acts as an inoculating mechanism for non-indigenous species such as viruses and bacteria, Dinoflagellate, diatoms and other protists, zooplankton, benthic fish, as well as eggs, spores, seeds, cysts and larvae of various aquatic plants and organisms.

The ballast water discharged from ships acts as an inoculating mechanism for non-indigenous species such as viruses and bacteria, Dinoflagellate, diatoms and other protists, zooplankton, benthic fish, as well as eggs, spores, seeds, cysts and larvae of various aquatic plants and organisms.

In addition, zooplankton, especially copepods, may be a carrier of pathogenic bacteria, such as *Vibrio cholerae* and *Vibrio alginolyticus* [2].

In February 2004, International Maritime Organization (IMO) adopted a regulation – "Convention for the Control and Management of Ships' Ballast Water and Sediments (Ballast Water Convention)". It regulates the methods of unloading ships' ballast water [3,7].

The convention refers to the reduction in the risk of non-indigenous species from sea ballast waters [4,5,6], and the main aim of the convention is to establish a Ballast Water Management System. The Ballast Water Management System shall solve the problem of uncontrolled intake and operations related to ballast water discharges in the period between 2009 and 2016.

In the future, instead of the existing system of ship's ballast water exchange, a complete transition to a system of ballast water treating is planned, which means that the ship's ballast water will be treated in accordance with standard rules of D-2 Ballast Water Convention before they are discharged into the marine environment [4, 7, 8].

D-2, a ballast water quality standard, requires that in a cubic meter of discharged ballast water should not be more than 10 surviving organisms that are equal as or greater than 50 μm, and in one milliliter should not be more than 10 surviving organisms whose dimensions are between 10 and 50 μm [9].

This standard also refers to the pathogens that represent a potential threat to human health. The standard is governed by the general health standards and sets a maximum number of colony forming units (cfu) per hundred milliliters of water for the three types of chosen indicator microbes, as follows [7, 8]: toxicogenic *Vibrio cholerae* (1 cfu/100 ml, or 1 cfu per gram of zooplanktonic sample, *Escherichia coli* (250 cfu/100 ml) and intestinal enterococci (100 cfu/100 ml).

The treatment of ballast water on ships is carried out by using the technologies that are integrated into the ballast system from the intake through the tanks down to the discharge. Thus the ballast water treatment can be performed during intake or discharge at the inlet/outlet, in the pipes or in the ballast tanks during navigation [10].

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According to the IMO Convention, ballast water treatment technologies should be [11, 12,13]: (1) safe, (2) environmentally friendly, (3) feasible, (4) cost-effective, and (5) biologically effective.

Very few of the existing ballast water treatment technologies meet all the five criteria of the IMO Convention. These are, for example [7]: UV irradiation, Ultrasonic treatment, Ozonation, SeaKleen technology, Deoxygenation, Cavitation, etc. Still, not one of these technologies is fully satisfactory. Thus, we decided to combine interdisciplinary experience of our research group to possibly find a new and acceptable solution that will meet the mentioned criteria of the IMO Convention.

The functioning of new technology is based on a combination of filtration, hydrocyclones and cavitation. The aim of experiments is to examine so far unexplored phenomenon of the appearance of hydrodynamic cavitation within the hydrocyclone. They will also try to prove the effectiveness of these combinations of technologies in removing marine organisms from ballast water.

Very few of the existing ballast water treatment technologies meet all the five criteria of the IMO Convention.

Filtration is used as the first step and the primary procedure of ballast water treatment in the new system (ship's filter with a grate 8 * 8 mm in diameter). The filtration eliminates organisms and waste of larger dimensions. Filtered water, with a help of the pumps, comes into the hydrocyclone, which uses centrifugal force to separate particles and organisms denser than the water density. They are eliminated through the lower exit of the hydrocyclone.

Hydrocyclones are inertial devices that enable separation or concentration of macrofluids as a suspension due to differences between the inertial forces that manage the movement of suspended substances in a liquid cargo [14]. The basic mechanism of hydrocyclone operation is the swirling flow, which influences the creation of centrifugal force [10, 15]. Sea water and organisms it contains do not have the same density.

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Organisms and sediment with a density greater than the density of water are suppressed by the swirling flow towards the wall of the hydrocyclone. They will glide down the wall and at the end of the process they will be ejected through the bottom outlet. The phase with less density, i.e. purified ballast water, remains in the central part, where, affected with the internal vortex, passes through the upper exit [16].

Figure 1 shows appearance and parts of the hydrocyclone device.

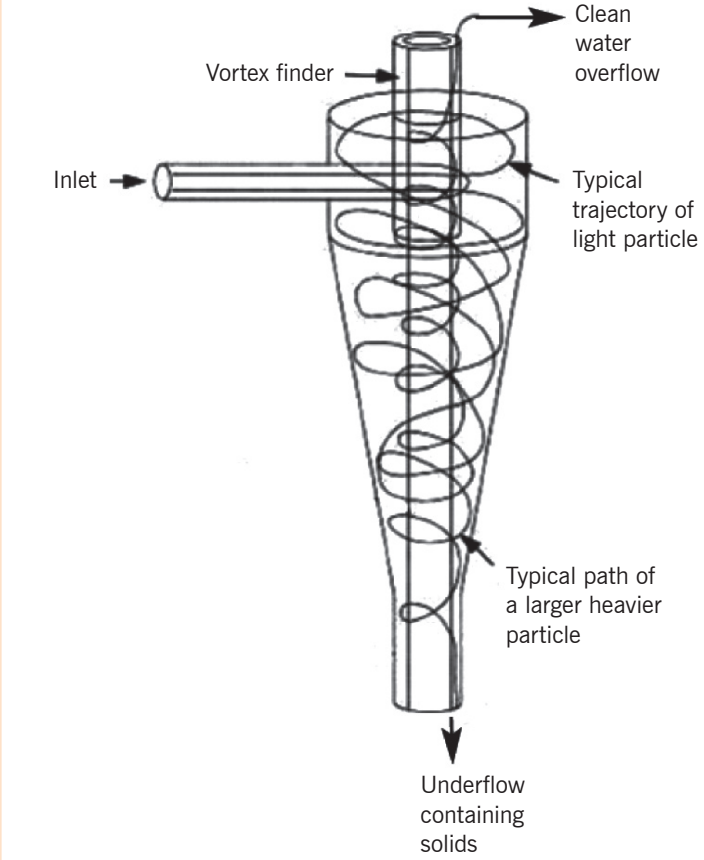

Figure 1:
Appearance and parts of the hydrocyclone device, (Source: Lloyd's Register. Ballast water treatment technology Current status. London, 2010.)

Hydrocyclones are considered to be a sustainable technology for the treatment of ballast water because of the simplicity of use, operation and maintenance, low power consumption, the possibility of working with a high water flow and resulting in a significant reduction of the problems associated with sedimentation in tanks. It is important to mention the fact that they do not have any impact on the health or safety of the ship and its crew.

Cavitation represents the phenomenon of creation, growth and collapse of micro bubbles in a fluid. When a certain volume of fluid is exposed to a sufficiently low pressure, the fluid can burst and form a cavity (cavitation) [17]. Soon after this burst, the vapour collapses back into the fluid - in this phase very high pressures and temperatures may be achieved on a micro scale.

The effects of hydrodynamic cavitation on chemical/physical processes and transformations are particularly investigated in the past decade [18]. The main reason for the development of hydrodynamic cavitation is a variation of pressure in the fluid flow, whereby vapor cavities can be formed anywhere in the liquid flow [19].

So far, hydrodynamic cavitation has been successfully applied for water disinfection, enzyme recovery and waste water treatment [20, 21]. Hydrodynamic cavitation can be scaled up for operation on very large scale, especially as required for ballast water treatment [17].

The main reason for the development of hydrodynamic cavitation is a variation of pressure in the fluid flow, whereby vapor cavities can be formed anywhere in the liquid flow.

Our idea is that hydrodynamic cavitation within the hydrocyclone should destroy the remaining organisms, i.e. the organisms whose density is equal as or less than the density of water and have escaped the centrifugal separation in the hydrocyclone. This step would also be the third (final) phase of operation of the new device for the ballast water treatment.

Until now, the use of these technologies for the ballast water treatment has been relatively well known and researched, one by one. Also, the combination of filtration and hydrocyclone technology is well known, and the application of these technologies in removing marine organisms from ballast water achieves high efficiency. But until now there are no reports on the proposed combination of filtration, hydrocyclone and hydrodynamic cavitation.

What is most important is the fact that the technologies proved their environmental acceptability; they are safe and economical without harmful chemical reactions or consequences to humans and environment.

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Sedna's system is one of the ballast water treatment systems that uses a combination of hydrocyclones and fine filtration (50 μm), together with a chemical agent Paraclean Ocean [22]. This ballast water treatment system has shown 98 % efficiency.

The technologies which have also combined in their work the use of hydrocyclones and filtration, and which, at the same time, meet the regulation D-2 of the IMO Ballast Water Convention are: ERMA FIRST S.A [23] (a combination of hydrocyclone, filtration and electrolytic cell for the extraction of chlorine to destroy the remaining organisms) and Hamworthy Greenship B.V. (a combination of hydrocyclone, filtration and electrolytic chlorination) [24].

Although successful application of a combination of filtration and hydrocyclones in the ballast water treatment has been scientifically proved up to now, the fundamental problem of existing technologies is the last stage of technology operation.

Although successful application of a combination of filtration and hydrocyclones in the ballast water treatment has been scientifically proved up to now, the fundamental problem of existing technologies is the last stage of technology operation. It has always involved the use of chemicals which means the increase of risk for humans and environment.

Also, the use of chemicals further increases the overall cost of technology. There is a risk of corrosion or other harmful impacts on materials, and there is a need for specific additional training of the crew on handling the technology.

The foreseen characteristics of our proposed new ballast water treatment technology are:

- No harmful effects on the environment
- Cost of technology (low power consumption, the use of relatively cheap materials, ease of maintenance, ease of handling)
- Low cost of purchase and device installation
- Universality of application in relation to the size and purpose of the ship, and the capacity of ballast tanks

– Relatively short time of treatment with new technology
– High percentage of efficiency in operating the technology
– No risk of corrosion
– Does not release toxic compounds and it is not hazardous in reaction with other substances
– Adjustable technology in terms of space (does not take up a large area)

METHODS

Description of laboratory pilot device

The pilot device consisted of a chamber, integrated by the cylindrical and conical parts (Figure 1). The cylindrical part of the chamber was made of Plexiglas material due to experimental needs, while the other parts were made of steel. The hydrocyclone was connected to the centrifugal pump.

The laboratory pilot device consisted of a hydrocyclone whose dimensions are shown in Table 1.

Table 1: Dimensions of laboratory hydrocyclone

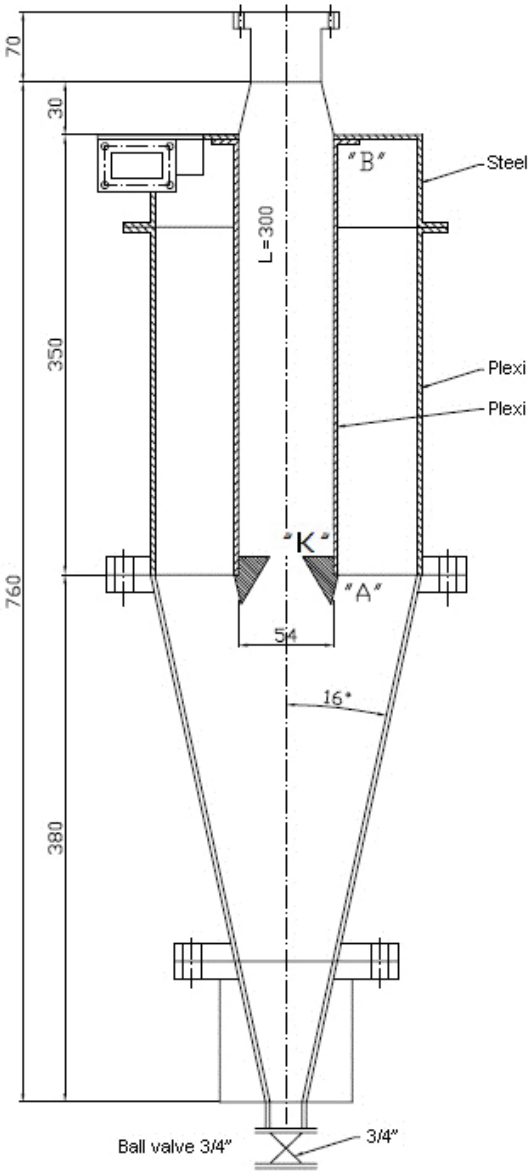
Width	18 cm
Length	36 cm
Cone length	32 cm
Cone angle	16°
Pressure	0,5-2 bar

The pilot device was constructed in such a manner that at the entrance of the vortex finder the phenomenon of hydrodynamic cavitation occurred. In order to achieve the best possible effect, in the first experiment, the entrance of the vortex finder, at the inlet section, had two conical mouths. A phenomenon of cavitation occurred at the “K” point of the cone.

In the second cycle of testing, the nozzles with holes of various diameters were set up at the end of the vortex finder (photo 1), while the third cycle of research included experiments with cross-shaped additions to the nozzles with 6 or 8 partitions mounted at the end of the vortex finder or inside the vortex finder. The additions to vortex finder were used as mechanisms for calming down the vortex and increasing the longitudinal speed of the fluid which resulted in increased cavitation.

In the experiments, two variations of vortex calming crosses were used – the first combination used in the experiments was a nozzle with a cross which was placed in the vortex finder of the hydrocyclone, while in the second variation, the cross was placed at the exit of the vortex finder as an inner extension of the vortex finder. The third variation used

Figure 2:
Schematic representation of the laboratory pilot device



in the experiments implied a combination of the last two mentioned systems.

Namely, the purpose of this segment of the experiment was to demonstrate the theoretical assumption that there was an increase of fluid velocity on the outer end of the vortex finder, which was caused by the placing of obstacles in different diameters, with the aim of creating rapid constrictions.

Photo 1:
The appearance of nozzles for hydrocyclone vortex finder

Therefore, the main goal was to increase the velocity of the fluid on the outer end of the vortex finder, with the aim of decreasing the pressure and, at the same time, creating the conditions for hydrodynamic cavitation occurrence.

The inflow of water into the hydrocyclone had a rectangular cross section, positioned tangentially with respect to the outer surface of the hydrocyclone. The ball valve 3/4" at the bottom (bilge) controlled the flow of waste water (sediments and majority of organisms) on exiting the hydrocyclone and returning them back into the sea.

The methods used and the further development of research

At this stage of research, a pilot device (hydrocyclone) was constructed, and the behavior of water flow within the hydrocyclone was monitored. With the aim of development of hydrodynamic cavitation phenomenon on the vortex finder, the nozzles with 8,12,14,16 and 20 mm diameter were tested, and also one nozzle with 19 holes 4 mm in diameter, and a combination of cross-shaped additions to a nozzle with 6 or 8 compartments that were placed at the end of or inside the vortex finder of the hydrocyclone.

With every change of a nozzle, or a nozzle with addition, the following factors were measured: flow rate, the pressure at the inlet ($p1$), the pressure before reduction ($p2$), the pressure at the entrance of the hydrocyclone ($p3$), the pressure of the hydrocyclone bilge ($p4$), the pressure at the exit of the hydrocyclone ($p5$), the pressure on the outer rim of the hydrocyclone ($p6$), the pressure on the inner rim of the hydrocyclone after the occurrence of hydrodynamic cavitation ($p7$), the pressure at the point of hydrodynamic cavitation ($p8$), the velocity of water in the vortex finder, the speed of water when passing through the nozzle (theoretical value).

The inflow of water into the hydrocyclone had a rectangular cross section, positioned tangentially with respect to the outer surface of the hydrocyclone.

In this phase, the hydrocyclone of corresponding characteristics was constructed, and the behavior of water flow within the hydrocyclone and the occurrence of phenomenon of the hydrodynamic cavitation and fluid motion trajectory were monitored, too.

In the next step of the research, the samples of sea water will be taken and the properties, the content and the presence of certain organisms in the sample before and after the treatment with new technology should be checked.

Further research will be divided into three groups:

1. Treatment of phytoplanktonic species;
2. Treatment of cysts and nauplii
3. Treatment of zooplanktonic species

The next phase of research should focus on checking whether the results obtained during experiments meet the quality standards of ballast water regulation D2, BWP (Ballast Water Performance).

The final step of the research of new ballast water treatment technology will be a trial testing of the effectiveness of the technology on board. The objective of this phase of the project is to examine the previously tested technology in real conditions, when the technology is integrated on the ship.

3. RESULTS AND DISCUSSION

The results of laboratory experiments with a pilot unit, where the nozzles of different diameters as well as the nozzles with combination of additions were used, are shown in the Table 2. Experiments were performed at the air temperatures of 20-21 °C, and the water temperature 19.32 °C.

An explanation for the nozzle and channel shapes:

0 - Normal nozzle with a hole

1 - Nozzle 0 with a cross for the vortex calming down

2 - Nozzle 0 with a cross for the vortex calming down positioned on the vortex finder

3 - Nozzle 1 with a cross for the vortex calming down positioned on the vortex finder

The basic parameter that describes the process of cavitation is a cavitation number. It is calculated by the following equation [26]:

$$\sigma = \frac{p_0 - p_v}{\frac{1}{2}\rho V^2}$$

Where: ρ is the density of the fluid, p_0 characteristic pressure, p_v is the vapor pressure of the liquid, V is a characteristic velocity of the flow.

Table 2: Results obtained from the experiments on a laboratory pilot unit

Nozzle	Form of nozzles and channels	Flow Q0		S nozzle	v nozzle (teoret.)	v outlet pipe	σ (cavit. No.)
ø [mm]		[m³/h]	[l/s]	[x 10⁻³ m²]	[m/s] (teoret.)	[m/s]	
12	0	2,77	0,769	0,113	6,803	0,367	10,187
12	2	2,72	0,755	0,113	6,680	0,361	8,144
12	3	2,62	0,727	0,113	6,434	0,348	10,899
14	0	4,57	1,269	0,154	8,246	0,607	4,927
14	2	4	1,111	0,154	7,217	0,531	5,177
14	3	5,96	1,655	0,154	10,754	0,791	**2,658**
16	0	4,55	1,263	0,201	6,286	0,604	12,142
16	1	5,55	1,541	0,201	7,667	0,737	5,087
16	2	5,4	1,5	0,201	7,460	0,717	6,468
16	3	8,88	2,46	0,201	12,268	1,179	**2,305**
20	1	8,92	2,477	0,314	7,887	1,184	4,539
19x 4	1	8,5	2,361	0,238	9,889	1,129	3,855
19x 4	3	8,34	2,316	0,238	9,702	1,107	3,973

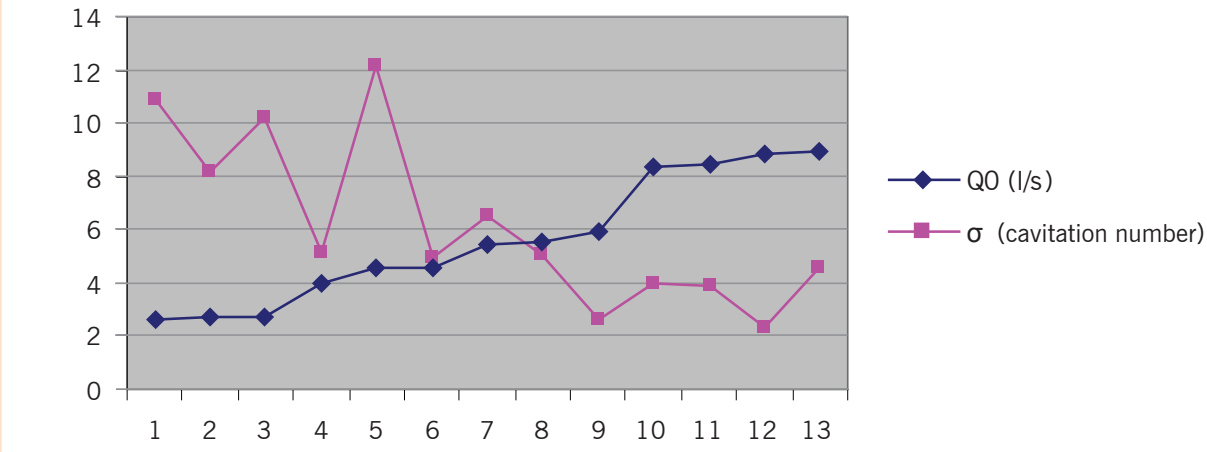


Figure 3:
Dependence of the flow Q_0 (l/s) on cavitation number σ

With the decrease of cavitation number σ the possibility of cavitation occurrence increases. If σ decreases below 2.5, the cavitation will appear. As the mentioned number decreases and approaches number one, cavitation will be getting stronger and stronger. When cavitation number is greater than one, it means that the fluid is resistant to cavitation. When cavitation number is less than one, it means that fluid energy (velocity head and pressure head at constriction) is being taken for the creation of vapor phase and hence cavitation [17].

Figure 2 shows the experimental results that describe the connection of hydrodynamic cavitation and flow in the pilot device. The graph shows the increasing flow in the laboratory hydrocyclone, which consequently influenced the decrease of cavitation number in the pipes (namely, with the increase of the flow rate, the velocity of fluid consequently increases, too). The results showed that the increase of the flow in the hydrocyclone had influenced the increase of the possibility of cavitation occurrence.

As evident from Table 2, the best efficiency (ie the strongest cavitation) was achieved during the usage of the nozzle 16 mm in diameter, and the addition of a cross for the vortex calming down, that was placed on the vortex finder and had a flow rate of 2.47 l / s

Figure 4:
Dependence of cavitation number σ on the diameter of a nozzle

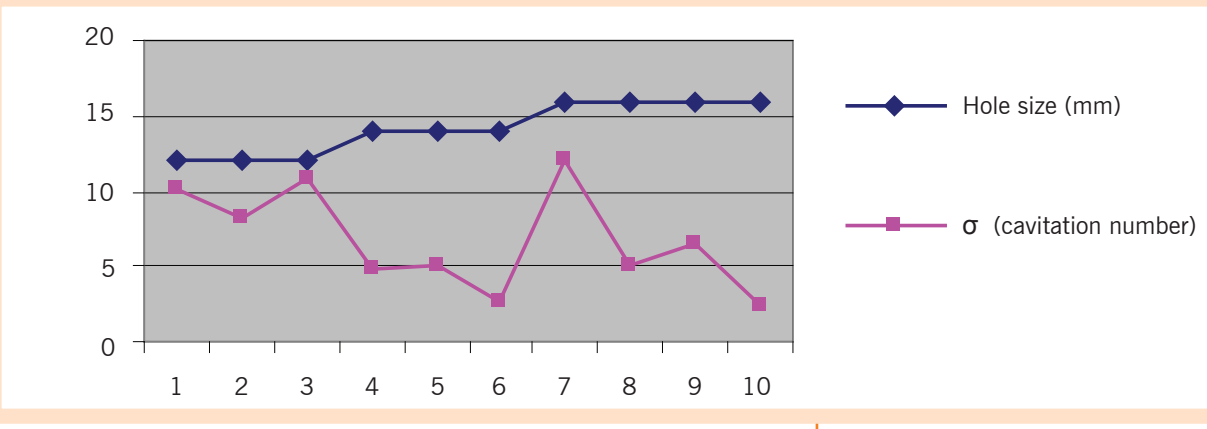


Figure 3 shows the dependence of cavitation number on the diameter of the nozzle mounted on the vortex finder of hydrocyclone. As evident from the graph, the most powerful hydrodynamic cavitation occurred during the usage of a nozzle 16 mm in diameter and with an additional cross for the vortex calming down, set on the vortex finder.

According to the results of the research, a very important effect on the occurrence of cavitation, other than a nozzle diameter, was the determination of proper direction and calming down the water vortex which was executed by using the cross for calming down placed on the vortex finder.

Table 3 and Figure 4 show the theoretical value of losses and pressure drop within a laboratory hydrocyclone for the following flows: 1.39 l / s,

Table 3: Energy losses and pressure drop as a function of changes in the flow of the pilot device

Q0	***d* nozzle**	**$\Delta v2/2g$**	**Δp**
(l/s)	***(x 10^{-2} m)***	**m**	**bar**
1,388	2,5	-0,385	-3,78 E-2
1,388	2	-0,974	- 9,56 E-2
1,388	1,6	-2,412	-2,36 E-1
1,388	1,4	-4,130	-4,05 E-1
1,388	1,2	-7,671	-7,52 E-1
1,388	1,1	-10,875	-1,066
1,388	1	**-15,932**	-1,562
1,666	2,5	-0,555	-5,45 E-2
1,666	2	-1,403	-1,37 E-1
1,666	1,6	-3,473	-3,4 E-1
1,666	1,4	-5,948	-5,83 E-1
1,666	1,2	-11,047	-1,083
1,666	1,1	-15,66	-1,536
1,666	1	**-22,942**	-2,250
1,944	2,5	-0,756	-7,42 E-2
1,944	2	-1,910	-1,87 E-1
1,944	1,6	-4,727	-4,63 E-1
1,944	1,4	- 8,096	-7,94 E-1
1,944	1,2	-15,036	-1,475
1,944	1,1	-21,314	-2,091
1,944	1	-31,227	-3,063
2,222	2,5	-0,988	-9,69 E-2
2,222	2	-2,495	-2,44 E-1
2,222	1,6	-6,174	-6, 05 E-1
2,222	1,4	-10,574	-1,037
2,222	1,2	-19,639	-1,926
2,222	1,1	-27,839	-2,731
2,222	1	**-40,787**	-4,001

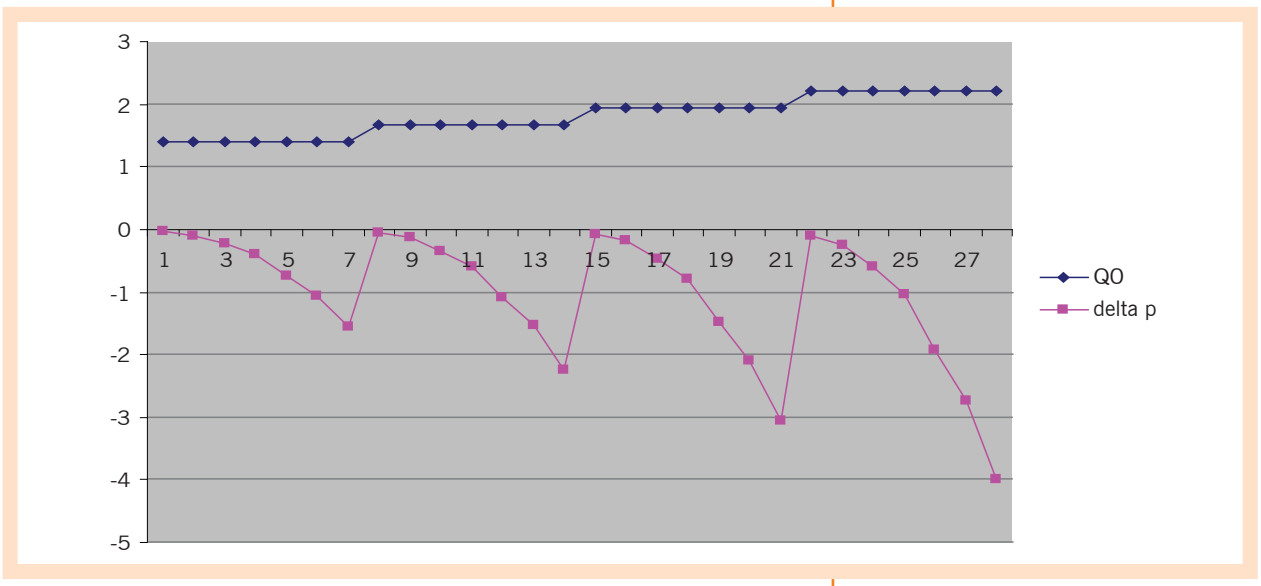


Figure 5:
Dependence of pressure drop on flow rates at different nozzle diameters

1.67 l / s, 1.94 l / s, 2.22 l / s (in the case of experiments with these flows, there was the strongest appearance of hydrodynamic cavitation).

The losses that occurred within the laboratory hydrocyclone increased proportionally with an increasing flow and with a reduction of the nozzle's diameter, as evident from the data given in Table 3. Thus, in these cases, for the smallest nozzle diameter of 0.01 m, (for all the tested flow cases samples), energy losses were the greatest in comparison with other measured flow rates and nozzle diameters.

According to Borda Carnot - equation of losses and pressure drop, depend on the following factors [25]: the density of medium and the square of the change of speed in the system.

According to Figure 4, where the pressure drop dependence on the flow rates of different diameters is shown, the largest pressure drops were noticed in the experiments with a nozzle which had the smallest diameter. This was directly connected with a flow increase. Namely, fluid velocity increases rapidly with the increase of flow and the reduction of the nozzle diameter. There was a sudden pressure drop which was approaching vapor pressure, and it consequently led to the formation of cavitation.

With the aim of the additional reduction of energy losses in further laboratory experiments, one of the possible changes could be the construction change in the structure of the laboratory pilot device.

It has been theoretically proven that if the length of the cylindrical portion (part of plexia) is reduced, than subsequently, for the same used input factors, the losses in pilot hydrocyclone system will be significantly reduced.

Table 4 shows a comparison of losses for the current length of 0.3 m for the cylindrical part of the laboratory hydrocyclone, and if the same was reduced to 0.12 m. Used flow rates were: 1.388 l / s, 1.666 l / s, 1.944 l / s, 2.222 l / s.

4. CONCLUSION

With the aim of achieving a high degree of efficiency in the removal of micro- and macro-organisms from sea water, reducing negative impacts on humans and environment, and satisfying economic criteria, the new ballast water treatment technology has been designed.

The functioning of new technology is based on the use of a combination of mechanical and physical ballast water treatment systems, and the innovation which this technology has brought is the causing of the appearance of otherwise undesirable phenomenon - hydrodynamic cavitation. It has been used for mechanical destruction of marine organisms that survived the previous step, hydrocyclonic treatment.

Former investigations made on the laboratory hydrocyclone have shown the occurrence of hydrodynamic cavitation on the outer edge of the Vortex Finder. Experiments have confirmed the thesis of the interdependence of hydrodynamic cavitation and other parameters such as flow, velocity in the pipe, and the speed in the hydrocyclone vortex finder. The occurrence of the strongest cavitation has been theoretically and experimentally proved during the usage of an addition to hydrocyclone's vortex finder in a shape of a nozzle 16 mm in diameter and a cross for calming down placed on the outer edge of the vortex finder. During the mentioned process, flow rate was 2.47 l/s.

Experiments have confirmed the thesis of the interdependence of hydrodynamic cavitation and other parameters such as flow, velocity in the pipe, and the speed in the hydrocyclone vortex finder.

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