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ORIGINAL PAPER

Biosensor-based comparison of the ecotoxicological contamination of the wastewaters of Southern Russia and Southern Germany

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Abstract To assess the ecotoxicological and sanitary situation in two European metropolis, Rostov-on-Don (Southern Russia) and Munich (Southern Germany), wastewaters of the two cities were examined with a panel of bacterial lux-biosensors: *Vibrio aquamarinus* VKPM B-11245, *Escherichia coli* MG1655 (pXen7), *E. coli* MG1655 (pRecA-lux), *E. coli* MG1655 (pSoxS-lux), *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pIbpA-lux), *E. coli* MG1655 (GrpE-lux), *E. coli* MG1655 (pFabA-lux). The presence of different genotoxic compounds and substances with the oxidative and membrane-damaging effects was revealed in contaminated wastewater with the applied panel of the lux-biosensors. The integral toxicity was approximately the same in both cities but demonstrated opposite trends. The presence of genotoxicants and peroxides was higher in the majority of the Munich wastewater samples. There were also differences in the presence of individual toxicants. The presence of the genotoxic compounds might also promote development and dissemination of several antibiotic resistance traits found in microorganisms, a feature more pronounced in Rostov-on-Don wastewaters. By means of polymerase chain reaction assay, antibiotic resistance genes to such antibiotics as *ermB*, *vim* and *vanB* were revealed in two Munich samples. Antibiotic resistance genes were present at all Rostov samples, and genes *ndm*, *vanA*, *vanB* and *ermB* were found. Taken together, the proposed analytical approach with the application of the constructed panel of biosensors can be applied for monitoring of the ecotoxicological contamination in the wastewaters of large cities.

Keywords Antibiotic resistance genes · Cell membrane damage · Genotoxicity · Municipal sewage · Prooxidant effect

Introduction

Industry development, growth of the cities and population lead to increase in both water consumption and water disposal (Figueras and Borrego 2010). Influx of a large amount of poorly purified sewage deteriorates the ecological and epidemiological quality of water ecosystems (Poma et al. 2012).

Effluents from sewage treatment plants are sources of a wide range of chemicals entering the aquatic environment (Sturve et al. 2008). As a result of industrial activity and household usage, a mixture of chemicals including PAHs, solvents, heavy metals, plasticisers, pharmaceuticals, flame retardants, antioxidants and washing and cleaning-related compounds is dumped into sewage (Paxéus 1996; Halling-Sorensen et al. 1998).

The high number of pollutants in sewage (Stadler et al. 2012; Tang et al. 2013) leads to the need for ecotoxicological assessment of water quality. Fast and effective methods are necessary for toxicity assessment. They include evaluation of such toxicity parameters as integral toxicity, genotoxicity and oxidative stress. As a rule, their detection is quite complicated to perform as a big amount of substances can cause similar effects (Tang et al. 2013). The chemical analysis that initially prevails in toxicity

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assessment of sewage, determines components and concentration of the polluting substances in the environment. However, it is unable to reveal the mechanisms of their toxic effects and the impact of pollution on living organisms (Ma et al. 2014).

One of the most promising approaches used for the environment monitoring is the application of biosensors (Daniel et al. 2008; Palchetti and Mascini 2008; Sorensen et al. 2006), including those based on bioluminescent bacteria (Ren 2004; Woutersen et al. 2011; Elad et al. 2011, 2013; Ji et al. 2013; Zhang et al. 2013; Ding et al. 2015; Eltzov et al. 2015).

Usage of lux-biosensor batteries provides an opportunity to evaluate the presence of different toxic substances in ecosystems simultaneously: DNA-tropic compounds (Ptitsyn et al. 1997; Vollmer et al. 1997; Biran et al. 2010), heavy metals (Lyngberg et al. 1999; Hakkila et al. 2004; Ivask et al. 2009), polychlorinated biphenyls (Layton et al. 1998), substances causing oxidative stress (Lee and Gu 2003, Zavilgelsky et al. 2007), damaging proteins (Van Dyk et al. 1994, 1995) and membranes (Choi and Gu 1999), etc. Besides, there is an opportunity to make a preliminary conclusion about the mechanism of their action.

The ability of many compounds to damage the genetic material of living organisms deserves special attention. The presence of the genotoxicants might significantly increase mutation rates and support rapid development and dissemination of such unwanted features as antibiotic resistance genes (ARG) number increase in microorganisms (Harwood et al. 2001; Iversen et al. 2002; Schwartz et al. 2003; Sahlström et al. 2009; Munir et al. 2011; Korzeniewska and Harnisz 2013; Amos et al. 2014).

Polymerase chain reaction (PCR) of the total DNA extracted from sewage or active sludge from waste treatment facilities can be successfully applied to follow the presence of ARG (Guillaume et al. 2000; Szczepanowski et al. 2009). It is known that traditional methods of sewage treatment are not effective enough and both effluents and active sludge of sewage treatment plants contain ARG and antibiotic resistant bacteria in significant amounts (Munir et al. 2011).

Accordingly, combining toxicity testing by means of bacterial biosensors and investigating ARG presence in sewage will allow obtaining a complex assessment of biological safety and quality of the municipal sewage.

The approach proves to be helpful in comparison with sewages of two large cities of Western and Eastern Europe and supplies with information on ecotoxicological parameters and presence of several determinants of resistance and bacterial contamination of their wastewaters. Wastewaters of the Rostov-on-Don and Munich were selected as the subject of the study. Studied wastewaters were collected at wastewater treatment plants of the Rostov-on-Don and Munich during the years 2012–2013. Munich and Rostov-on-Don are similar according to the number of inhabitants ($\sim$1,300,000 and $\sim$1,100,000 people), but considerably differ from the viewpoint of the living standards and of the population mobility.

Materials and methods

The present research was carried out on wastewaters.

Site of collection

The studied wastewaters were collected at municipal wastewater treatment plants of the Rostov-on-Don and Munich.

Sampling

Wastewater samples were taken at wastewater treatment plants, at the stage of microbiological purification on October 05, 2012; December 03, 2012; May 29, 2013; and July 08, 2013. A portion (500 ml) of each sample was packed in sterile chemically clear containers and sent to the laboratory. In the laboratory, it was subsampled into 50 ml aliquots and stored at −20 °C until analyzed.

Bacterial strains and culture conditions

Strains *Vibrio aquamarinus* VKPM B-11245 (*Vibrio aquamarinus* DSM 26054), *E. coli* MG1655 (pXen7-lux), *E. coli* MG1655 (pRecA-lux), *E. coli* MG1655 (pKatG-lux), *E. coli* MG1655 (pSoxS-lux), *E. coli* MG1655 (pIbpA-lux), *E. coli* MG1655 (pFabA-lux) were used in this study. Strains are kindly provided by I.V. Manukhov (Federal State Unitary Enterprise "GosNIIGenetika").

Strain *Vibrio aquamarinus* VKPM B-11245 was isolated by us from Black Sea water. It has high sensitivity for toxicants and used for analysis of general toxicity (Sazykin et al. 2014). The strain is more sensitive compared to the recombinant *Escherichia coli* strain with the cloned lux-operon *P. leiognathi*, used in the test system « Ecolum » (Methods 2007; Deriabin and Aleshina 2008).

The biosensor with *PrecA* promotor fixes the presence of the factors causing damage of DNA in a cell. The biosensor with the *PkatG* promoter fixes production of hydroperoxides in a cell and with the *PsoxS* promotor-superoxide anion and NO (Vollmer et al. 1997; Belkin et al. 2003; Lee and Gu 2003; Zavilgelsky et al. 2007; Lushchak 2011). Biosensor strain with *PibpA* promotor responds to the substances damaging proteins (Van Dyk et al. 1994, 1995). Biosensor strain with *PfabA* promotor

responds to the substances damaging membranes (Choi and Gu 1999).

Bioluminescent strains were obtained by transformation of *E. coli* MG1655 by hybrid plasmids pXen7, pRecA-lux, pKatG-lux, pSoxS-lux, pIbpA-lux, pFabA-lux. The gene cassette *luxCDABE Photorhabdus luminescens* under the control of *Plac*, *PrecA*, *PkatG*, *PsoxS*, *PibpA*, *PfabA* promoters, respectively, was used in these biosensors. These plasmids are created on the basis of pBR322 and contain a selective marker of ampicillin resistance (*Amp* gene).

The bacterial strains were cultivated in Luria–Bertani (LB) medium (Maniatis et al. 1982), containing 100 μg of ampicillin/ml. The cultures were grown under constant shaking to early exponential phase at 37 °C. Cells were used immediately for stress induction tests.

Chemicals

All of the chemicals used were of analytical grade. Hydrogen peroxide was from "Ferraine." Methyl viologen, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) ("Sigma-Aldrich") and $ZnSO_4$ were obtained from «Sigma-Aldrich». Pentachlorophenol, glucose-6-phosphate, NADP were obtained from "AppliChem." Test solutions were prepared in distilled water immediately before the tests. Rat liver microsomal enzymes (S9 fraction) were from "Moltox."

Biosensors assay procedure

Wastewater samples to be tested were added in 20-μl portions to wells of a 96-well microplate containing 180 μl of the culture. In the control, 20 μl of distilled water was added. Twenty microliters of toxicant solution (in case of positive control for promoter activation) was introduced into other wells.

For control activation of the *PsoxS* promoter, methyl viologen was used, for *PkatG* promoter activation—hydrogen peroxide, for *PrecA* promoter activation—*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (« Sigma »), for *PIbpA* promoters—high temperature (50 °C, 5 min), for *PfabA* promoter—pentachlorophenol. As positive control for *E. coli* MG1655 (pXen7), zinc sulfate was used.

When determining genotoxicity in an embodiment with metabolic activation (designated in the tables as "+S9"), 160 μl of culture, 20 μl of the water sample (in the control variant—20 μl of distilled water) and 20 μl of activating mixture comprising S9 fraction of rat liver microsomal enzymes ("Moltox," USA) were added to the wells.

Luminescence measurements were taken on microplate luminometer LM–01T ("Immunotech"). Numerical values of a bioluminescence were expressed in relative luminescence units.

Calculation

The criterion of toxic influence is bioluminescence intensity change of the test object in the researched sample in comparison with the control sample.

Strong toxic influence of the studied toxicant on bacteria is evaluated according to the inhibition of their bioluminescence for 30-min exposition period.

The quantitative assessment of the test reaction parameter is reflected as a dimensionless number—the toxicity index (T), calculated according to the formula:

$$T = 100\,(I_k - I_c)/I_c,$$

where I_c and I_k are the intensity of bacteria luminescence in proof and control samples, respectively, at fixed exposition time of the studied solution with test object.

In some cases, a situation is possible when bioluminescence intensity of an analyzed sample is higher than that of the control sample. In that case irrespective of the size of negative T value, the conclusion about the absence of the sample toxicity is drawn, and the toxicity index equals zero.

The technique allows three threshold levels of the toxicity index:

- Admissible degree of toxicity: The toxicity index is less than 20.
- The sample is toxic: The toxicity index is equal or more than 20 and <50.
- The sample is highly toxic: The toxicity index is equal or more than 50.

All the experiments were carried out in three independent replications.

The induction factor, F_i, was defined as the relation of luminescence intensity of a lux-biosensor suspension, containing tested sample (L_c), to the luminescence intensity of a lux-biosensor control suspension (L_k): $F_i = L_c/L_k$. When the degree of luminescence induction is evaluated in environmental samples, it should be noted that many of the substances included in their composition, can enhance and suppress bacterial bioluminescence, influencing the bacterial luciferase enzyme that can cause artifacts. To solve this problem, the isogenic *E. coli* MG1655 (pXen7) lux-operon is under the control of a constitutive promoter which was used to correct the artifacts associated with changes in luciferase activity.

Therefore, besides, the induction factor coefficient of luminescence suppression (K) was determined: $K = l_c/l_k$, where l_c—luminescence intensity suspension lux-strain with constitutive promoter in the presence of the test compound; l_k—luminescence intensity control suspension lux-strain with constitutive promoter.

The correct values of the induction factor were calculated using the formula $I = F_i/K$, where F_i—induction factor, K—coefficient of luminescence suppression.

Difference reliability of bioluminescence in experiment from control value was estimated by t-criterion with the help of Excel program. The conclusion about sample toxicity was made at $p < 0.05$.

If at significant differences from control induction factor values were <2, the detected genotoxic effect was evaluated as « weak » , and if they were in the range from 2 to 10—as « medium » , above 10—as « strong » . All the experiments were carried out three times independently.

DNA extraction from samples of sewage

Forty milliliters of each sewage sample was centrifuged for 7 min at 8000*g*, 4 °C (Allegra X-30R centrifuge, Beckman Coulter, USA), and the sediment was suspended in 800 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and transferred into 2-ml Eppendorf tubes. Then, 200 µl 10 % of SDS was added to resuspend the sediment and carefully mixed. The suspension was incubated for 30 min at 99 °C, incubated with 250 µl of 5 M NaCl solution and carefully mixed. Then, it was centrifuged for 5 min at 14000*g*, at room temperature (MiniSpin plus centrifuge, Eppendorf, Germany), and the supernatant was transferred to a new 2-ml Eppendorf tubes. To the supernatant, 750 µl of the isopropanol was added; the mixture was allowed to dissolve for 10 min at +4 °C and then centrifuged 7 min at 14,000*g*. The sediment was washed twice with 70 % ethanol and dissolved in deionized water.

PCR assay

Commercial available PCR kits from "Litekh" (Moscow, Russia) were used for PCR assay. Resistance to carbapenems (genes vim, ndm, oxa-48), cephalosporins (genes ctx-M и mecA), glycopeptides—vancomycin and teicoplanin (genes vanA and vanB) and erythromycin (gene ermB) was discovered in sewage samples. Amplification was carried out in 0.2-ml PCR test tubes. In total, 20 µl of PCR mix and 5 µl of DNA from sewage were introduced into the tubes. PCR assays were carried out according to the protocol of the manufacturer using the T-100 amplifier ("Bio-Rad," USA). Amplification products were detected by horizontal electrophoresis. The presence of a band corresponding in size to inner control included into the PCR mix proved the success of the amplification process. The presence of antibiotic resistance genes was confirmed by the presence of a band corresponding to positive control included into each PCR kit.

Electrophoresis of the obtained amplicons was carried out in 1,2 % agarose gel, in 0,5X TBE buffer (0.54 g/l Tris base, 0.275 g/l boric acid, 1 mM EDTA, pH 8.3) for 1 h at 7 V/cm in the SE-2 camera for horizontal electrophoresis ("Helikon," Russia).

Results and discussion

A battery of bacterial lux-biosensors was applied to assess the toxicity of Rostov-on-Don and Munich wastewaters, and the pollution dynamics within the studied time period was evaluated. The use of a battery of bioluminescent tests can indicate general stress as a result of complex mixtures and provide information about the real risk to water environment.

The data presented allow the comparison of pollutants presence in wastewaters of the two cities (Table 1). Values of the bioluminescence induction factor (F_i) of bacterial lux-biosensors on the basis of *E. coli* MG1655 were obtained after diluting wastewaters 1: 100 due to the high toxicity of wastewaters, which does not allow to evaluate luminescence induction of bacterial sensor strains adequately (Eltzov et al. 2015). A natural biosensor strain *Vibrio aquamarinus* demonstrated even higher sensitivity to the integrated toxicity, in comparison with lux-biosensors on the basis of *E. coli*, and thus, the samples were diluted 1:1000.

According to the data presented in Fig. 1, the maximum integrated toxicity in Rostov-on-Don wastewaters was observed in October 2012 and that of Munich wastewaters—in July 2013. In December 2012, May 2013 and July 2013, the toxicity index decrease was registered in wastewaters of Rostov-on-Don. On the contrary, the toxicity index was higher in the wastewaters of Munich. Thus, during the observation period, the Rostov and Munich wastewaters showed comparable values of integrated toxicity, but, simultaneously, oppositely directed trends.

Luminescent bacteria (*Vibrio fischeri*, *Photobacterium phosphoreum*, etc.) have been used for assessing the integral toxicity in ecological monitoring for many years. One of the results of their response is a change in light emission intensity depending on sample toxicity degree. Results from luminescent bacteria acute toxicity test proved to be a valuable tool for efficient wastewaters pollution control (Rodrigues and Umbuzeiro 2011; Kokkali and Delft 2014; Prasse et al. 2015).

For instance, it was shown by Palma et al. (2010) that *V. fisheri* luminescence suppression correlates with the general content of phosphorus, chlorine compounds and heavy metals. *P. phosphoreum* was successfully used for determination of different molecular weight fractions of sludge treating synthetic wastewater containing 4-chlorophenol (Zhao et al. 2015). *V. fischeri* was used for assessment of ecotoxicity of mobile forms of heavy metals in sewage sludge (Gondek et al. 2014).

Table 1 Bioluminescent response of bacterial lux-biosensors to Rostov-on-Don and Munich wastewaters

Date and location of sampling	The toxicity index (T)	The induction factor (I)					
	Vibrio aquamarinus VKPM B-11245	*E. coli* MG1655 (pRecA-lux)		*E. coli* MG1655 (pKatG-lux)	*E. coli* MG1655 (pSoxS-lux)	*E. coli* MG1655 (pIbpA-lux)	*E. coli MG1655* (pFabA-lux)
		−S9	+S9				
October 05, 2012							
Rostov-on-Don	20.83*	1.97*	1.24	1.43	2.28*	2.75*	3.38*
Munich	2.86*	2.16*	1.32	2.57*	1.36	2.98*	3.21*
December 03, 2012							
Rostov-on-Don	14.01*	1.30	1.69*	2.47*	2.39*	1.10	2.58*
Munich	7.12*	2.31*	1.65*	2.66*	2.10*	1.59*	2.69*
May 29, 2013							
Rostov-on-Don	1.22*	1.71*	1.64*	1.71*	1.21	2.13*	2.10*
Munich	8.86*	2.90*	2.86*	1.25	1.24	1.73*	3.15*
July 08, 2013							
Rostov-on-Don	2.86*	1.64*	2.36*	1.59*	1.15	2.49*	2.03*
Munich	19.63*	2.82*	2.71*	2.04*	2.58*	1.83*	4.03*

* Differences compared to the control samples are statistically significant, t-criterion, $p < 0.05$. Metabolic activation samples are marked +S9, without activation −S9

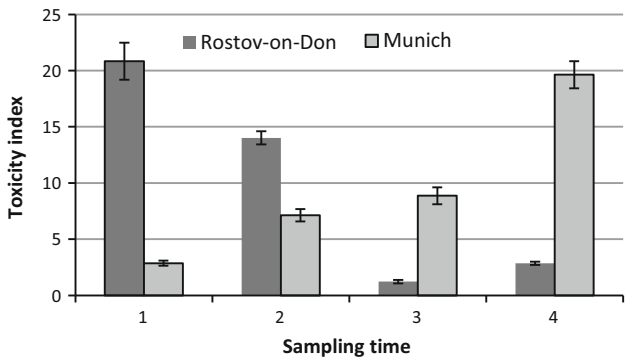

Fig. 1 Integral toxicity of Rostov-on-Don and Munich wastewaters (strain *Vibrio aquamarinus* VKPM B-11245): *1*—October 2012; *2*—December 2012; *3*—May 2013; *4*—July 2013

The dump filtrates behave likewise (Bhalla et al. 2013). The concentration of dissolved substances and toxicity of wastewaters increase during the periods of plentiful rainfall and were demonstrated with bacterial lux-biosensors and toxic influence on amphibian larvae (Palma et al. 2010; Park et al. 2014).

June and July are supposed to be the rainiest months in Munich. At the same time, there is an efficient storm drainage system directing wastewaters into the general collectors and further to the wastewater treatment facilities in Munich. In contrast, in Rostov, there is almost no storm drainage and the most part of rainfall flows directly to the Don River. The maximum rainfall in Rostov is in June and from December to January with October being one of the driest months of the year.

Detection of DNA damage is the most essential toxicity effect that can be discovered with the help of bioluminescent bacteria strains. Genotoxicity assessment is of great importance for public and environmental health; therefore, genotoxicity testing is among the most widely used bioassays in ecotoxicology (Prasse et al. 2015).

Escherichia coli MG1655 (pRecA-lux) strain was used for genotoxicity assessment. Comparison of the induction factors determined with the *E. coli* MG1655 (pRecA-lux) biosensor showed that Munich wastewaters contain a larger amount of direct genotoxicants in winter, spring and summer samples (Fig. 2). During spring and summer, the increase in the induction factor was observed for promutagen substances using metabolic activation (Fig. 3) and direct mutagens (Fig. 2) in Munich wastewaters.

In Rostov wastewaters, on the contrary, the concentration of the direct mutagens and promutagen compounds was the highest in October and in July, correspondingly. One can assume, due to the high levels of pollution in spring and summer in Munich, both direct mutagens and promutagens stream into wastewaters simultaneously, perhaps as part of the same pollutants. The trend toward increase in genotoxicity corresponds with the growth of the integrated wastewaters toxicity in Munich during the observation period. In contrast, in Rostov, there were two maximum genotoxicant inflows separated by an interval of 8 months; one—in October 2012, the inflow of direct genotoxicants, and another—in July 2013—the inflow of

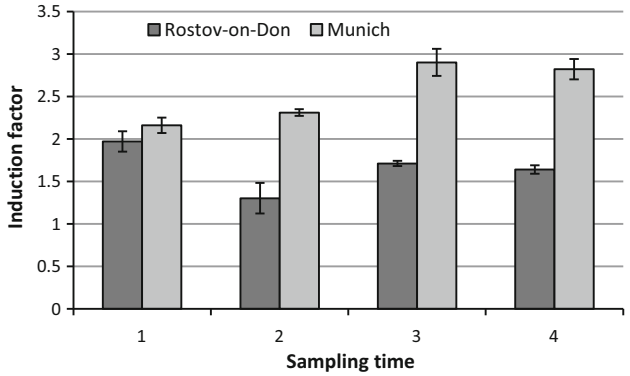

Fig. 2 Wastewaters genotoxicity: *E. coli* MG1655 (pRecA-lux) biosensor response without using metabolic activation: *1*—October 2012; *2*—December 2012; *3*—May 2013; *4*—July 2013

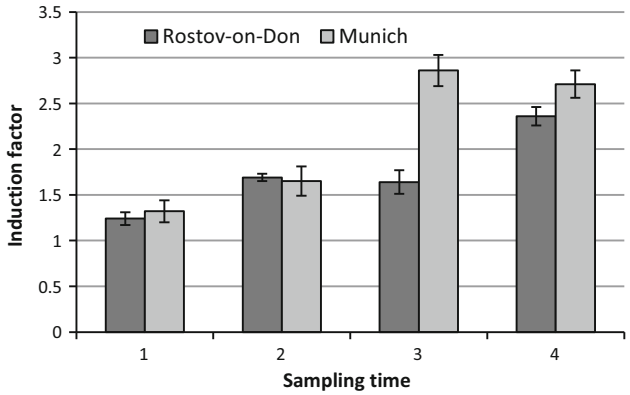

Fig. 3 Wastewaters genotoxicity: *E. coli* MG1655 (pRecA-lux) biosensor response using metabolic activation: *1*—October 2012; *2*—December 2012; *3*—May 2013; *4*—July 2013

promutagens. Moreover, the promutagens increase was linked with the fall of the integrated wastewaters toxicity, potentially indicating changes of the composition of the pollutants.

Many of the wastewater compounds are known to be toxic to organisms due to their ability to form reactive oxygen species and cause oxidative stress (Sturve et al. 2008). Therein, they are also able to cause critical effects such as oxidative damage to lipids and proteins (Livingstone 2001; Carney Almroth et al. 2008). Consequently, using the parameters of oxidative stress in monitoring of environmental pollution has increased in recent years and has a great significance as an early warning signal (Valavanidis et al. 2006).

In order to investigate the presence of compounds that may cause oxidative stress, *E. coli* MG1655 (pKatG-lux) and *E. coli* MG 1655 (pSoxS-lux) strains were used. *E. coli* MG1655 (pIbpA-lux) and *E. coli* MG1655 (pFabA-lux) sensor strains were used for detection of protein and membrane damages, correspondingly.

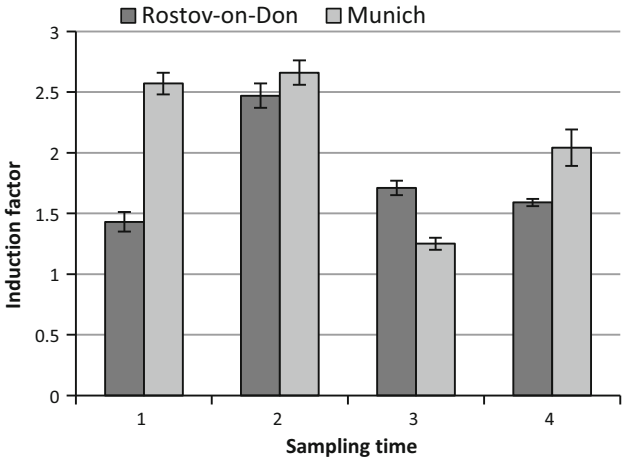

Fig. 4 Wastewaters prooxidant activity: *E. coli* MG1655 (pKatG-lux) biosensor response: *1*—October 2012; *2*—December 2012; *3*—May 2013; *4*—July 2013

A large amount of peroxides that cause oxidative stress was detected in Munich wastewaters in all time probes except for samples collected in May (Fig. 4). Their maximum concentration both in Munich and in Rostov wastewaters was observed in winter (possibly due to their greater stability at low temperatures and, as a consequence, their accumulation). It should be noted that the contribution of peroxides into integrated Munich wastewaters toxicity during the observation period decreased.

Escherichia coli MG 1655 (pSoxS-lux) biosensor response to the presence of substances that cause the superoxide anion formation was higher in the wastewaters of Rostov-on-Don in October 2012 and in the wastewaters of Munich in July 2013. Their maximum concentration in the wastewaters of Rostov occurred in October and December 2012 and in Munich wastewaters—in July 2013 (Fig. 5). The general trend toward reduction in concentration of substances causing oxidative stress (except October 2012 samples) in Rostov wastewaters correlates with the integrated toxicity decrease.

E. coli MG1655 (pIbpA-lux) sensor strain response to the presence of substances that cause protein damages was the strongest in the wastewaters of Rostov-on-Don and of Munich in autumn (see Fig. 6) and changed likewise over the period of observation in both cities. However, a relative contribution of the substances causing protein damage to the integral toxicity and the number of promutagens increased in Rostov wastewater that indicates qualitative changes in the wastewaters composition. In Munich, on the contrary, the contribution of the protein-damaging agents to the integral toxicity of wastewaters decreased during the whole observation period.

The response of the *E. coli* MG1655 (pFabA-lux) biosensor to the membrane-damaging substances was 1.5

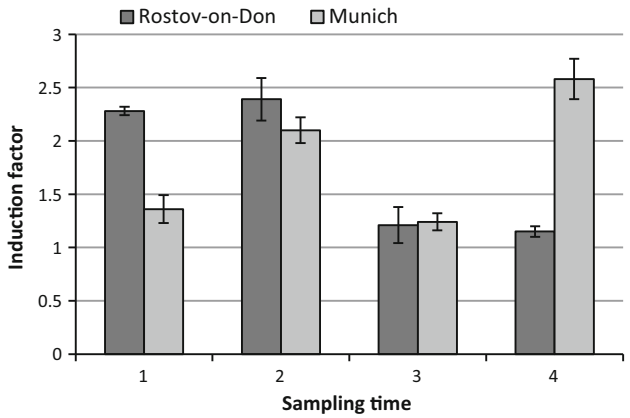

Fig. 5 Wastewaters prooxidant activity: *E. coli* MG1655 (pSoxS-lux) biosensor response: *1*—October 2012; *2*—December 2012; *3*—May 2013; *4*—July 2013

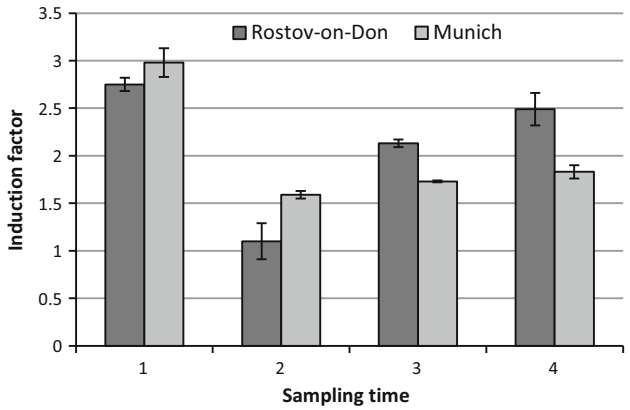

Fig. 6 Detection of protein-damaging substances in wastewaters: *E. coli* MG1655 (pIbpA-lux) biosensor response: *1*—October 2012; *2*—December 2012; *3*—May 2013; *4*—July 2013

and 2 times higher in Munich sewage in May and July 2013. The increase in the membrane-damaging substance concentrations was observed in Munich wastewaters during winter–summer period. Their presence decreased in Rostov wastewaters over the research period (Fig. 7). The changes in concentrations of the membrane-damaging substances (e.g., surfactants) largely correlated with the changes in the integral wastewaters toxicity of Rostov and Munich during the observation period. Thus, it can be assumed that the membrane-damaging agents were mainly responsible for the integral wastewaters toxicity. Genotoxicants of both direct and indirect action were the main toxic pollutants for Munich and substances that caused oxidative stress—for Rostov-on-Don.

The toxicity of Munich wastewaters seems to be primarily caused by the presence of membrane-damaging substances, genotoxicants and substances causing formation of superoxide anion (especially in the summer

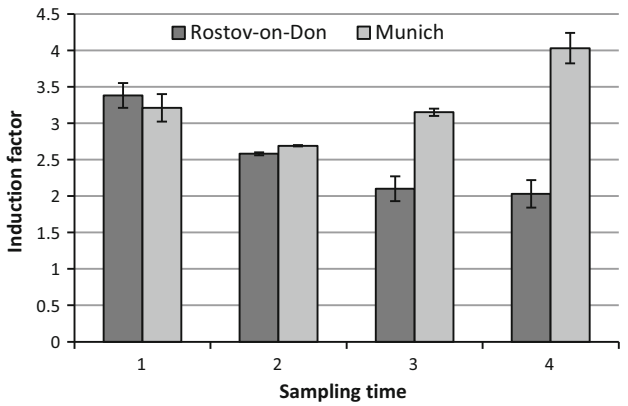

Fig. 7 Detection of membrane-damaging substances in wastewaters: *E. coli* MG1655 (pFabA-lux) biosensor response: *1*—October 2012; *2*—December 2012; *3*—May 2013; *4*—July 2013

sample). This is supported by the highest responses of the biosensors *E. coli* MG1655 (pFabA-lux), *E. coli* MG1655 (pRecA-lux) and *E. coli* MG 1655 (pSoxS-lux) along with the highest index of integrated toxicity of *Vibrio aquamarinus* VKPM B-11245, respectively. Most probably, the presence of the prooxidant substances can promote genotoxic effects, either by activating promutagens by oxidation or by direct DNA alkylation.

Integral toxicity of Rostov-on-Don wastewaters is also largely conditioned by cell membrane damage. Direct mutagens and substances that cause oxidative stress (primarily superoxide anion) also contribute to the toxicity. Thus, the similar mechanism of toxic action of municipal waste can be stated in these two cities.

PCR analysis of the total DNA isolated from sewage indicated the presence of genes confirming resistance to erythromycin (*ermB*) in Munich samples dating from December 03, 2012, and July 08, 2013, and the presence of *vim* genes (*Enterobacteriaceae* and *Pseudomonas* resistance to carbapenems) and *vanB* genes (*Enterococcus* resistance to teicoplanin) in a sample from December 03, 2012. However, in spring and autumn samples, none of the analyzed genes was identified. All the total DNA samples from wastewaters of Rostov displayed the presence of ARG: October 05, 2012—resistance to carbapenems (*ndm*), vancomycin (*vanA*) and teicoplanin (*vanB*); December 03, 2012—erythromycin (*ermB*); May 29, 2013—erythromycin (*ermB*), carbapenems (*ndm*), vancomycin (*vanA*) and teicoplanin (*vanB*); July 08, 2013—erythromycin (*ermB*) and teicoplanin (*vanB*).

Although even in Germany, only one-fourth of antibiotics can be associated with their use in hospitals (Kümmerer and Henninger 2004), the constant presence and a wide range of ARG in the Rostov-on-Don wastewaters,

compared to Munich, are probably due to the free public availability of antibiotics (OTC sales) and less control of the antibiotics usage in Russia.

ARG in Munich sewage were detected in winter and summer samples, which also caused the largest oxidative stress response in bacterial biosensors (SoxS-lux and KatG-lux). In Rostov sewage, on the contrary, the greatest variety of ARG was determined in autumn and spring samples. These samples showed a higher induction factor of the biosensor responding to direct mutagens (RecA-lux without metabolic activation). Both effects, detected with the biosensors, may be caused by biocides. Gaze et al. (2011) reported an increase in the number of mobile genetic elements with resistance genes after bacterial exposure to biocides and detergents. On the other hand, no influence of detergents has been observed; biosensor induction coefficient for membranes damage [*E. coli* MG1655 (pFabA-lux)] did not correlate with the presence of variety of ARG in sewage. Also no connection between the range of ARG and response level of the other biosensors was revealed.

Conclusion

A composite response of the applied biosensors panel allows not only to assess the presence of toxic substances in wastewaters of the two cities, but also to evaluate the toxic spectrum of pollutants in environmental monitoring of aquatic ecosystems. Taken together, the data obtained with the bacterial lux-biosensors demonstrate that the integral toxicity of wastewaters of both Munich and Rostov during the observation period was at the similar level but demonstrated opposite trends. The complex response of the bioluminescent sensors panel revealed that the toxicity of sewage of both Munich and Rostov-on-Don is mainly caused by the membrane-damaging substances. Direct genotoxicants and promutagens, as well as substances that cause oxidative stress, make a significant contribution to the integral toxicity of sewage. The Rostov wastewaters, according to expression of various toxicity mechanisms in different samples, were more susceptible to variations in their qualitative composition compared to Munich wastewaters.

A larger spectrum of ARG was detected in the wastewaters of Rostov-on-Don in comparison with those of Munich. Prooxidant compounds and direct genotoxicants, as well-known stressors, can directly increase the frequency of mutagenesis but also facilitate distribution of the mobile ARG-bearing elements already present in this epitope. This might directly influence the resistome of municipal wastewaters. The uncontrolled use of antibiotics and their availability in Russia should be kept in mind. However, the effect of membrane-damaging substances on the variety of ARG in municipal wastewaters was not found.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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