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Residues of selected sulfonamides, non-steroidal anti-inflammatory drugs and analgesics-antipyretics in surface water of the Elbe river basin (Czech Republic)

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Abstract: The occurrence of human as well as veterinary drug residues in surface water is caused by their insufficient removal ability from wastewater. Drug residues disturb the natural balance of water ecosystem, have a negative effect on non-target organisms and pose a significant risk for human health. The main aim of this study was to determine the concentration of residues of eight drugs from the group of sulfonamides (sulfathiazole, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfachlorpyridazine), four drugs from the non-steroidal anti-inflammatory drug group (ibuprofen, ketoprofen, naproxen, diclofenac) and one representative of the analgesics-antipyretics group [paracetamol (acetaminophen)] in the surface water of the Elbe river basin. A total of 65 samples of surface water from the Elbe river basin were taken during August 2018 when the weather was constant without any significant fluctuations. The analysis was performed by means of liquid chromatography with tandem mass spectrometry (LC-MS/MS). The results have shown the numerous occurrences of sulfamethoxazole, ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen). A statistically significant negative correlation between the river flow rate in the monitored locations and the residue concentration was found for ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen). The most significant findings of the monitored drug residues were mostly determined in samples from small streams below larger urban settlements with a hospital or other health facilities.

Keywords: diclofenac; ibuprofen; naproxen; paracetamol (acetaminophen); sulfamethoxazole

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Pollution of surface water by pharmaceutical residues is a worldwide problem (Hruska and Franek 2012) mainly caused by the growing consumption of human and veterinary drugs and their insufficient disposal in sewage treatment plants (STPs). Significant groups of drugs widely used in human and veterinary medicine are sulfonamides, non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics-antipyretics (Nikolaou et al. 2007).

Sulfonamides, as a representative group of antibiotics, are used in veterinary medicine mainly in the treatment of bacterial infections in pig and cattle farms (Hruska and Franek 2012). Due to the problematic degradability of sulfonamide residues, intensive pig and cattle farms pose a high ecological risk for surface water pollution (Zhou et al. 2009). In the environment, the residues of antibiotics allow the development of multidrug-resistant bacteria and treatment with standard pharmaceuticals becomes ineffective. The residues of sulfonamides may cause the bioaccumulation and biomagnification in aquatic organisms (Bai et al. 2014).

Non-steroidal anti-inflammatory drugs are very often used in human as well as veterinary medicine mainly for pain treatment (Nikolaou et al. 2007). They are a heterogenic group of compounds with analgesic, antipyretic and antiphlogistic effects. An antiplatelet and antiuratic effect in some of these drugs has also been described (Pavelka 2002; Olejarova 2008; Forejtova 2017; Martinkova et al. 2018).

There are many negative types of impact caused by the residues of non-steroidal anti-inflammatory drugs in the environment. The main example is diclofenac, which is responsible for a critical threat status in 3 species of vultures (*genus Gyps – Gyps bengalensis, Gyps indicus, Gyps tenuirostris*) in south Asia (Swan et al. 2006). The use of diclofenac in pain treatment protocols in livestock is very typical in this area. The vultures were exposed to diclofenac toxicity during the ingestion of carrion animals treated by diclofenac. The study confirms a correlation between the exposition to the drug and the renal failure in the vultures (Oaks et al. 2004).

Analgesics-antipyretics are a group of compounds frequently used in the treatment of pain and fever. Due to the insignificant antiphlogistic effect, analgesics-antipyretics are a separate group of pharmaceuticals with a typical compound paracetamol (acetaminophen); however, in some monographs, the analgesics-antipyretics are still included as a part of the NSAIDs (Gerrett 2005).

Pharmaceuticals enter the surface water in several ways. One of the main sources of the contamination of surface water by drug residues is waste from hospital facilities, production sites and especially municipal waste (Sukul and Spiteller 2006). The use of pharmaceuticals in aquaculture and the excretion of their residues through excrement leads to their accumulation in the sediment. The application of contaminated fertilisers and sewage from STPs into the soil in order to achieve sustainable nutrient recycling is another way the drug residues enter into the environment.

A part of the drug residues occurring in the contaminated excrements from outdoor livestock resorbs into the soil and the rest is washed away together with rainwater into the surface water (Hamscher et al. 2004).

The drug residues in the surface water are incorporated into the sediment or undergo abiotic or biotic degradation (Sukul and Spiteller 2006; Hruska and Franek 2012). The drug transfer is also known to occur in dust particles from feed, bedding, animal excrement and from the animals themselves (Hamscher et al. 2003).

The level of environmental pollution is influenced by many factors, for example, the amount of farm animals, the fertilisation technology, the drug selection, the soil type, etc. (Hruska and Franek 2012).

As a result of the insufficient disposal of the drug residues from STPs, they occur in surface water, where they have a negative effect on aquatic organisms and disturb the balance of the aquatic ecosystem (Stancova et al. 2015).

The antibiotic pollution of surface water was described for the first time in a study from England in 1982. All the rivers included in the study were contaminated with at least one of the compounds from macrolides, sulfonamides and tetracyclines in the concentration of 1 µg/l (Watts et al. 1982). The occurrence of NSAID residues in surface water were described for the first time in a study from Canada in 1986. Ibuprofen and naproxen residues were detected in the samples from the effluents from the STPs (Rogers et al. 1986).

The present study aimed to determine the concentrations of the selected drug residues in the surface water of the Elbe river basin (Czech Republic) and to evaluate the relationship between their concentrations and the river flow rates.

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MATERIAL AND METHODS

Chemicals and reagents

In the present study, the concentration levels of thirteen drugs inthe Elbe river basin were determined. Eight compounds of sulfonamides (sulfathiazole, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfadimethoxine, sulfadoxine, sulfamerazine and sulfachlorpyridazine), four compounds from the non-steroidal anti-inflammatory drugs (ibuprofen, ketoprofen, naproxen, and diclofenac), and one compound from the analgesic-antipyretic group [paracetamol (acetaminophen)]. Ultra-clear drug-free water (SG Ultra Clear, Hamburg, Germany) with total organic carbon (TOC) measurements was used for the mobile phase preparation, optimisation, validation of the method and calibration of the solution preparation.

Substances of purity suitable for the analytical standard of the selected sulfonamides, non-steroidal anti-inflammatory drugs, and analgesics-antipyretics at a concentration of 10 mg/ml were used to form individual pharmaceutical stock solutions. These were sulfadimethoxine, sulfapyridine, sulfamethoxazole, ibuprofen, diclofenac, ketoprofen, naproxen, paracetamol (Dr. Ehrenstorfer, Augsburg, Germany); sulfathiazole, sulfadoxin, sulfachloropyridine, sulfadiazine (Supelco, Bellefonte, PA, USA); sulfamethazine (European Pharmacopoeia, Strasbourg, France). All vials were tared and 10 mg of the substance was added with a narrow spoon and weighed to five decimal places and filled with 1 ml of pure methanol LiChrosolv® (Merck, Darmstadt, Germany) using a 1 ml syringe (Merck, Darmstadt, Germany). An analyte primary dilution standard (PDS) solution (10 µg/ml) was prepared from the individual stock solutions by adding 10 µl (10 µl Hamilton syringe) into a 10 ml volumetric flask with methanol.

The same approach was used for the preparation of a stock solution of internal standards with a concentration of 10 µg/ml. These were paracetamol-D4, diclofenac-D4, ibuprofen-D3, sulfamethoxazole-D4 (Toronto Research Chemicals, North York, ON, Canada).

Sample collection

The point water samples were taken from sixty-five locations along the Elbe river basin (Figures 1 and 2). The sample collection was carried out at once into one-litre glass jars with ground-glass stoppers and the samples were cooled during transport to the laboratory. The sampling was performed during August 2018, when the weather as well as the intensity of the river flow rates was constant.

Sample preparation

Once in the laboratory, the samples were filtered through regenerated cellulose (17 mm) syringe filter (Thermo Fisher Scientific, Waltham, MA, USA) with a 0.2 µm pore size into 15 ml clear glass vials with a polytetrafluoroethylene (PTFE) seal screw-top (Supelco, Bellefonte, PA, USA), which were consequently stored at 4 °C. After 24 h, 1 ml of each filtered sample was dispensed by a 1 ml syringe (Merck, Darmstadt, Germany) in two 2 ml TruView vials (Thermo Fisher Scientific, Waltham, MA, USA) with a slit PTFE/silicone septum for measurement in an autosampler. Both vials were prelabeled with the specific sample numeric code and was subsequently entered into the measurement spreadsheet of MassHunter computer software (Agilent Technologies, Santa Clara, CA, USA) with this code. Twenty-five µl of the internal standard stock solution was added to each vial with 1 ml of the sample. One of them was assigned a plus sign due to addition 10 µl of the working standard solution (10 µl of the solution at a concentration of 10 µg/l, the final concentration was 100 ng/l). Immediately after preparation, all the vials were capped and prepared for analysis.

LC-MS/MS analysis

The analysis of the drug residue concentration was performed by liquid chromatography with

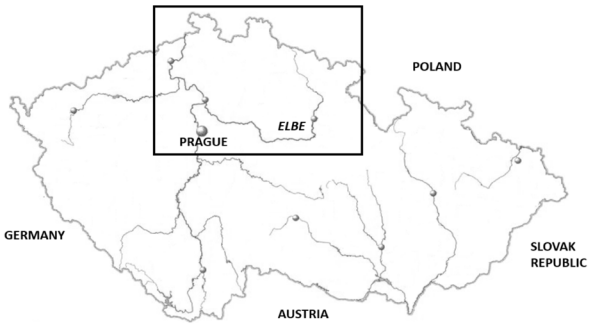

Figure 1. A map of the Czech Republic (the Elbe river basin)

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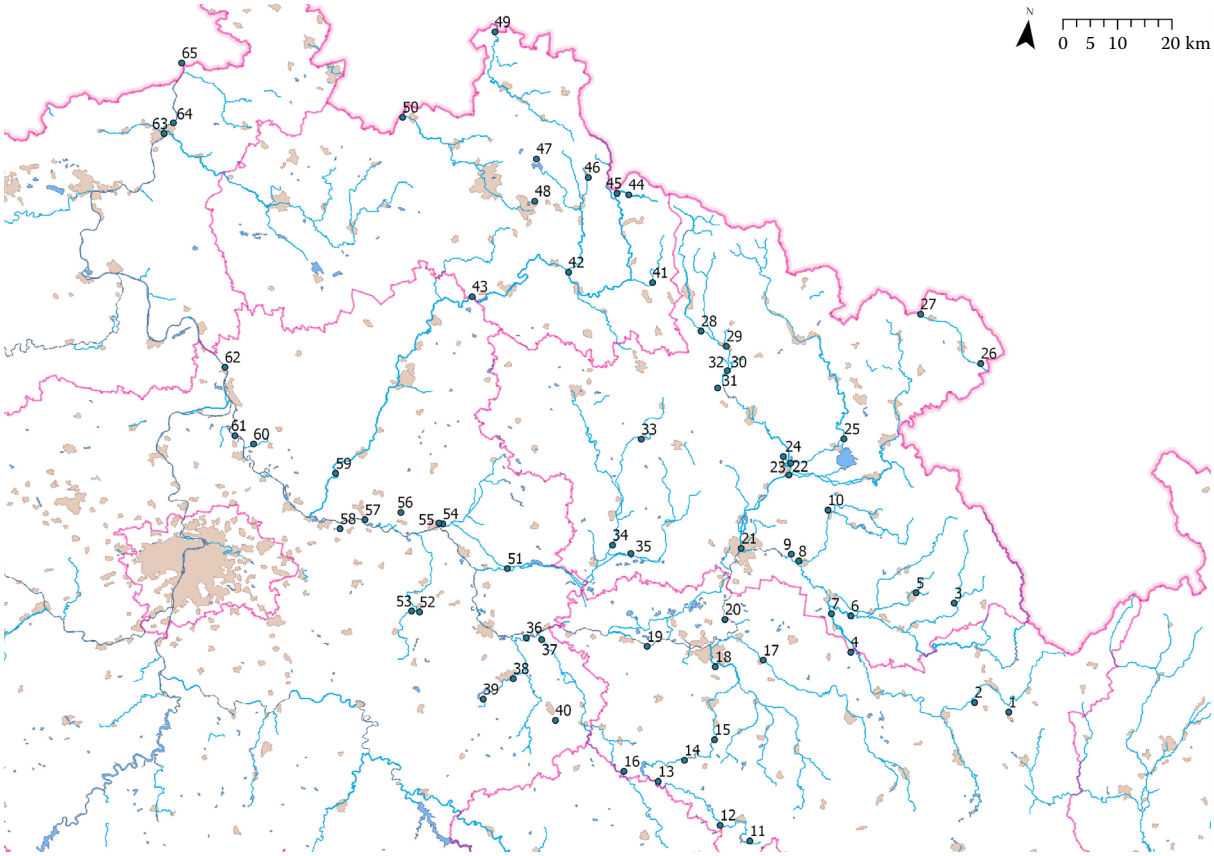

Figure 2. A map of sampling sites of the Elbe river basin

STP = sewage treatment plant

Explanations: Sample No. (river flow rate m^3/s): 1 – Čermná. Petrovice u Lanškrouna; 2 – Dobroučka. Dolní Dobrouč; 3 – Zdobnice. Pěčín (1.27); 4 – Čermná. Malá Čermná nad Orlicí (2.59); 5 – Javornický potok. Rychnov nad Kněžnou (0.24); 6 – Divoká Orlice. Čestice (4.26); 7 – Tichá Orlice. Žďár nad Orlicí (3.94); 8 – Dědina. Třebechovice pod Orebem (0.893); 9 – Orlice. Nepasice (8.78); 10 – Zlatý potok. České Meziříčí (0.22); 11 – Chrudimka. Silnice nad Hlinskem (0.21); 12 – Chrudimka. Stan (0.385); 13 – Chrudimka. Klokočov (1.001); 14 – Chrudimka. Mezisvětí (1.027); 15 – Chrudimka. Svídnice (0.86); 16 – Doubrava. Spačice (0.368); 17 – Loučná. Dašice (0.658); 18 – Chrudimka. Nemošice (1.7); 19 – Labe. Valy (26.62); 20 – Labe. Němčice (22); 21 – Labe. Hradec Králové (21.8); 22 – Metuje. Jaroměř (1.612); 23 – Úpa. Jaroměř (1.14); 24 – Labe. Hořenice (8.66); 25 – Olešnice. Zlíč (0.279); 26 – Stěnava. Otovice (0.69); 27 – Stěnava. Starostín (0.22); 28 – Labe. Klášterská Lhota (2.966); 29 – Čistá. Hostinné (0.59); 30 – Labe. Debrné (4.04); 31 – Borecký potok. Souvrať (0.56); 32 – Kalenský potok. Debrné (0.232); 33 – Javorka. Ostroměř (0.084); 34 – Cidlina. Luková (0.513); 35 – Bystřice. Kosičky (0.163); 36 – Labe. Veletov; 37 – Doubrava. Záboří nad Labem (0.42); 38 – Vrchlice. Kutná Hora (STP) (0.53); 39 – Vrchlice. Malešov (above reservoir) (0.32); 40 – Brslenka. Drobovice (0.28); 41 – Jizerka. Dolní Štěpanice (3.65); 42 – Kamenice. Spálov (1.71); 43 – Jizera. Příšovice (7.55); 44 – Mumlava. Harrachov (1.04); 45 – Mumlava. Mýtiny (2.05); 46 – Černá Desná (reservoir). Souš (0.043); 47 – Kamenice (above reservoir) (0.425); 48 – Lužická Nisa. Jablonecké Paseky (0.407); 49 – Smědá. Ves u Černous (0.972); 50 – Lužická Nisa. Hrádek nad Nisou (1.93); 51 – Cidlina. Sány (0.666); 52 – Bečvárka. Žabonosy (0.28); 53 – Výrovka. Zalešany (1.94); 54 – Mrlina. Nymburk (1.41); 55 – Labe. Nymburk (30); 56 – Vlkava. Hronětice (0.61); 57 – Labe. Lysá nad Labem; 58 – Výmola. Císařská Kuchyně (0.35); 59 – Jizera. Tuřice (2.35); 60 – Košátecký potok. Tišice (0.7); 61 – Labe. Obříství (42); 62 – Labe. Dolní Beřkovice – Liběchov (85); 63 – Labe. Děčín (130); 64 – Labe. Loubí; 65 – Labe. Hřensko/Schmilka (137)

the tandem mass spectrometry method (LC-MS/MS) with the LC-MS/MS instrument by Agilent Technologies (6495 Triple Quadrupole LC/MS System; Santa Clara, CA, USA). An Acquity UPLC HSS T3 Column, 1.8 µm, 2.1 mm × 100 mm (Waters, Milford, MA, USA) and an Acquity UPLC HSS T3 VanGuard Pre-column, 1.8 µm, 2.1 mm × 5 mm (Waters, Milford, MA, USA) were used to meas-

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ure the samples. The injection volume was 100 µl and the column temperature 40 °C. One analysis lasted 19 minutes.

The mobile phase consisted of two components. Component A was a mixture of 100% methanol LiChrosolv® (Supelco, Bellefonte, PA, USA) with 0.001% acetic acid [≥ 99% (Fluka Chemie GmbH, Buchs, Switzerland)]. Component B consisted of a mixture of water with 3% methanol LiChrosolv® (Supelco, Bellefonte, PA, USA) with 0.001% acetic acid ≥ 99% (Fluka Chemie GmbH, Buchs, Switzerland).

The working calibration solution was prepared by diluting the PDS solution (10 µg/ml) 1 : 1 000 into a 10 ml volumetric flask with water and adding 250 µl of the internal standard solution (10 µg/l).

The calibration standard solutions of the pharmaceutical mixtures (0, 5, 10, 20, 50, 100, 250, 1 000 and 2 000 ng/l) were prepared by diluting 10 µg/l of the solution using the appropriate volume Hamilton syringes into a 10 ml volumetric flask with water and 250 µl of the internal standard solution (10 µg/l).

The quantification limit was above 5 ng/l for all the monitored compounds except for paracetamol (acetaminophen), for which the limit was higher than 10 ng/l.

Statistical data

The statistical data processing was performed using Unistat for Excel v5.6 program. Firstly, the Shapiro-Wilk test was used to assess the normal distribution, which was not confirmed. Subsequently, the data were tested by using non-parametric tests. An evaluation of the correlation between the river flow and the concentration of the observed analytes was performed. For the correlation analysis, only data with a positive occurrence of the drug residues were used and the level of significance was $P \leq 0.05$, $P \leq 0.01$.

RESULTS

In the present study, residual concentrations of the following seven compounds: sulfadimidine, sulfamethoxazole, diclofenac, ibuprofen, ketoprofen, naproxen, paracetamol (acetaminophen), were found above the limit of quantification. While the residues of sulfadimidine and ketoprofen were measured in only 3 sampling sites, the residual concentrations of the remaining 5 compounds [sulfamethoxazole, ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen)] were found in most sampling sites. The correlation analysis showed a statistically significant correlation between the river flow rates and the concentration of the drug residues of ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen).

Sulfamethoxazole (Figure 3) was determined in a total of 48 samples and the highest concentration (270 ng/l) was measured in the sample from the Cidlina river in the village Luková (smp. 34). A sample from the river Výmola in the village Císařská Kuchyně (smp. 58) contained residues of sulfamethoxazole at the concentration of 190 ng/l and significant concentrations (140 and 130 ng/l) were also measured in the rivers Lužická

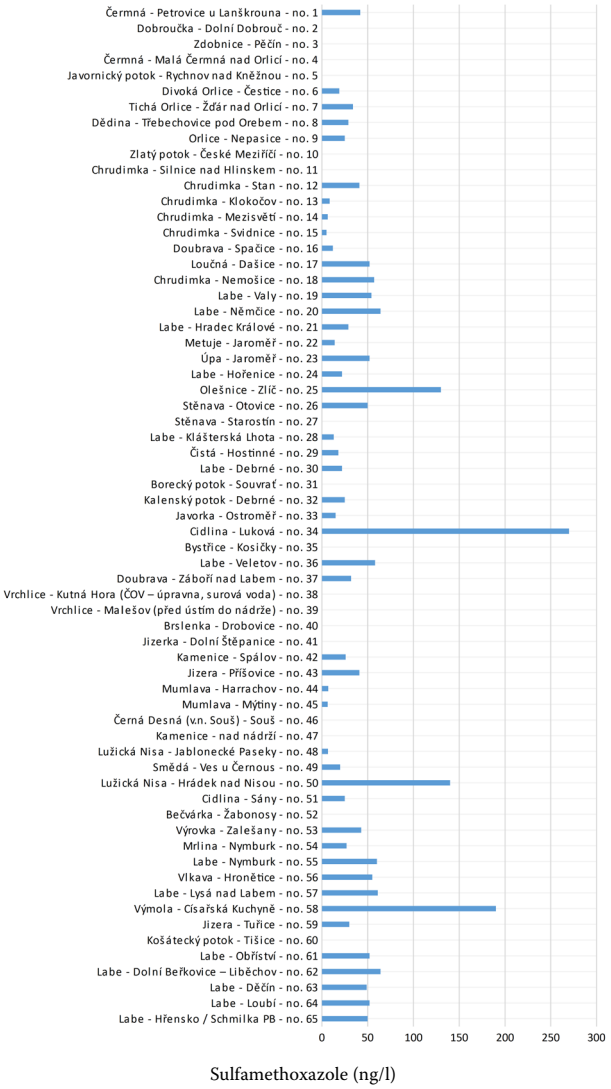

Figure 3. Concentrations of the sulfamethoxazole residues

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Nisa in Hrádek nad Nisou (smp. 50) and Olešnice in Zlíč (smp. 25). The correlation analysis did not show any statistically significant negative relation between the river flow rate and the concentration of sulfamethoxazole (P = 0.138 5).

Ibuprofen (Figure 4) was found in 43 samples. The highest concentration (1 600 ng/l) was in the sample from the Cidlina river in the village Luková (smp. 34). Significant concentrations (530 and 280 ng/l) of ibuprofen were determined in the samples from the Chrudimka river in a part of village Vítanov – Stan (smp. 12) and from the Borecký stream in Souvrať (smp. 31). The correlation analysis confirmed a statistically significant negative correlation between the river flow rate and the concentration of ibuprofen residues (P = 0.001 9).

Naproxen (Figure 5) was found in 19 samples and reached the highest concentration (160 ng/l) in a sample from the Stěnava river in the village Starostín (smp. 27). A high concentration (110 ng/l) of naproxen was determined in a sample from the Borecký stream in the village Souvrať (smp. 31). Other significant values of naproxen (69 and 58 ng/l) were found in the samples from the rivers Cidlina in the village Luková (smp. 34) and Výmola in the village Císařská Kuchyně (smp. 58). A statistically significant negative correlation between the intensity of the river flow and the concentration of naproxen residues (P = 0.05) was detected.

Diclofenac (Figure 6) was represented in most of the samples (in a total of 51 samples) with the highest concentration being detected in a sample from the Výmola river in the village Císařská Kuchyně (smp. 58). Another high value (260 ng/l) of diclofenac was measured in a sample from the Cidlina river in the village Luková (smp. 34).

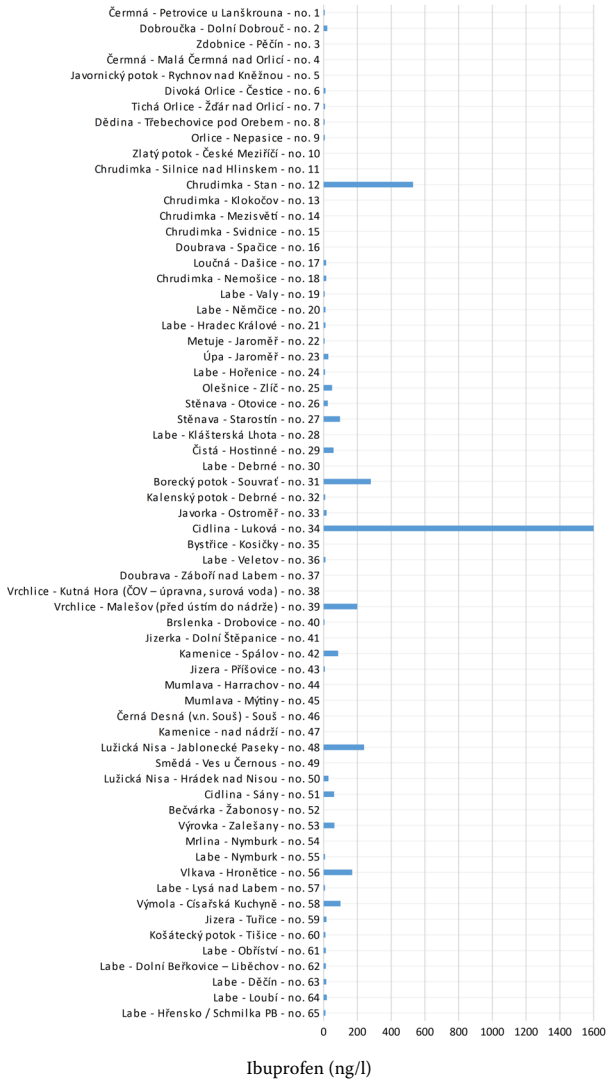

Figure 4. Concentrations of the ibuprofen residues

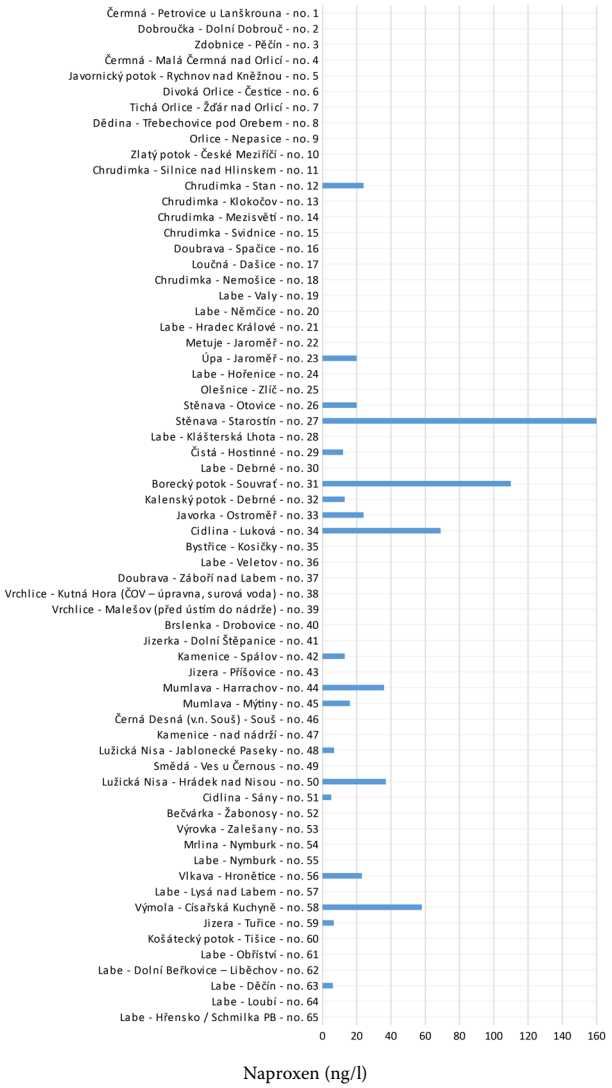

Figure 5. Concentrations of the naproxen residues

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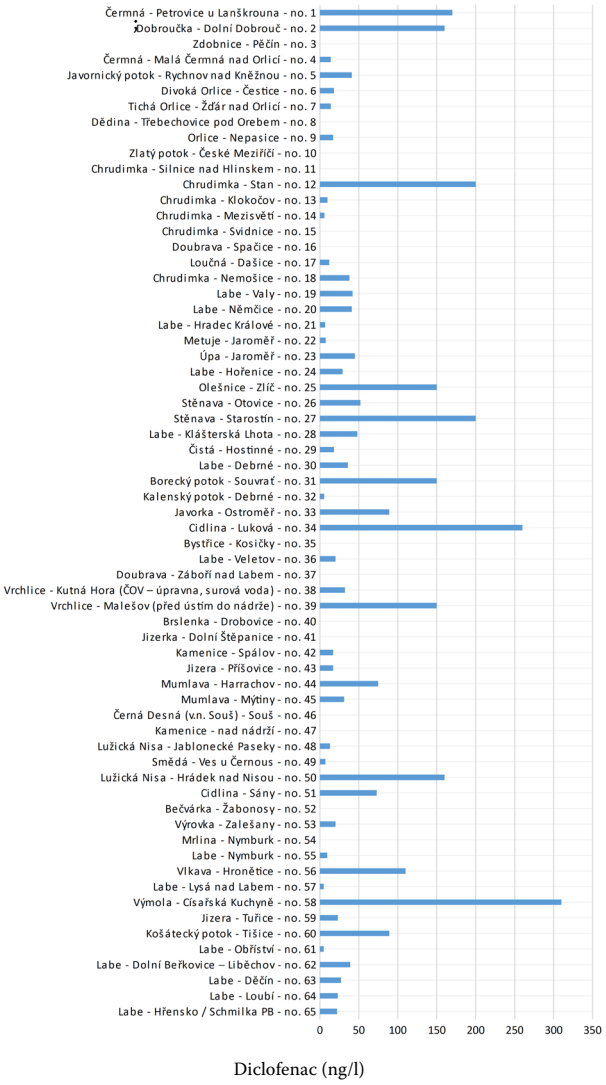

Figure 6. Concentrations of the diclofenac residues

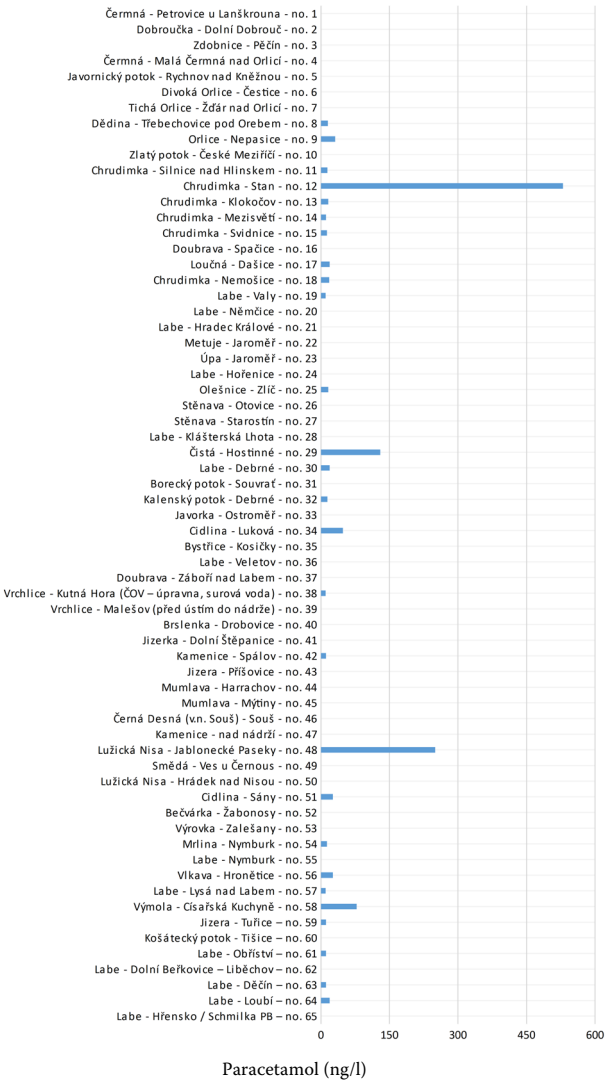

Figure 7. Concentrations of the paracetamol (acetaminophen) residues

Significant findings with the same concentration (200 ng/l) were detected in the samples from the Chrudimka river in a part of Vítanov – Stan (smp. 12) and the Stěnava river in the village Starostín (smp. 27).

A statistically significant negative correlation was confirmed between the intensity of the river flow and the concentration of diclofenac residues (P = 0.002 1).

Paracetamol, i.e., acetaminophen (Figure 7) was detected in 27 samples with the highest concentration (530 ng/l) in a sample from the Chrudimka river in a part of the village Vítanov – Stan (smp. 12). Other high concentrations (250 and 130 ng/l) of paracetamol (acetaminophen) were found in the samples from the Lužická Nisa river in the village Jablonecké Paseky (smp. 48) and the Čistá river in the village Hostinné (smp. 29). A statistically significant negative correlation between the intensity of the river flow and the concentration of paracetamol (acetaminophen) residues (P = 0.018 9) was confirmed.

DISCUSSION

The results show the occurrence of 7 drug residues in the samples of the surface water of the Elbe river basin. Significant concentrations in most locations were detected in the case of 5 drugs [sulfamethoxazole, ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen)]. An important part of the results is the confirmation of the statistically

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significant negative correlation between the river flow rate and the concentrations of the residues of ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen). However, no negative correlation between the concentrations of sulfamethoxazole residues and the river flow rates was demonstrated in this study. The values of the sulfamethoxazole residues were comparable in the most of the localities and oscillated around a concentration of 50 ng/l.

There were only four localities, where the concentrations of these residues were many times higher than the others. These localities were also abundantly polluted by the other monitored drug residues. Due to the nature of the results and the comparable concentrations, the correlation between the concentration of sulfamethoxazole residues and the river flow rates could not be clearly demonstrated. The low intensity of the flow in small streams leads to the minimal dilution of the drug residues and their concentrations are higher than in large rivers. The correlation between the river flow rate and the concentration of the drug residues is discussed in several studies (Ternes 1998; Hirsch et al. 1999; Kolpin et al. 2004; Kasprzyk-Hordern et al. 2008; Castiglioni and Zuccato 2011; Marsik et al. 2017; Veras et al. 2019).

The study by Castiglioni and Zuccato (2011) deals with the correlation of the drug residue concentrations in rivers with different flow rates. The observed rivers were the Po (600–1 000 m^3/s), the Lambro (5 m^3/s), the Olona (0.5 m^3/s) and the Arno (4–10 m^3/s). The Po, being the main river in northern Italy with a high flow rate, contained, in general, a lower concentration of drug residues due to the higher dilution. The authors Kasprzyk-Hordern et al. (2008) focused on studying the drug residues in the Taff (a large river) and the Ely (a small stream) in southern Wales in Great Britain. Due to a dry period, the flow rate was decreased, which caused the minimum dilution of the residues and it led to a significant increase in their concentrations in the streams. The study also observed the residues of sulfamethoxazole, ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen). Another study by Kolpin et al. (2004) discussed different concentrations of antibiotic residues depending on the flow rates in Iowa streams. The study compared streams with a low (F = 0.001), normal (F = 0.11) and high (F = 0.42) flow rate. The highest concentrations of antibiotic residues were determined under the low flow conditions when the dilution of the drug residues was only minimal. Hirsch et al. (1999) observed antibiotic residues including sulfamethoxazole. Higher concentrations of residues were detected in small streams compared to a large river, for instance the Rhine. Ternes (1998) states, in his study, that drug residues including ibuprofen and diclofenac occur in higher concentrations in small streams than in large rivers such as the Rhine and the Main. The authors Marsik et al. (2017) reported, in their study, that the concentrations of all monitored compounds except ibuprofen were significantly higher in the sampling sites with a low river flow rate (streams) than in large watercourses. The study by Veras et al. (2019) focused on the presence of NSAID residues at two sampling sites of the Beberibe river in Peixinhos (Brazil). The first sampling site was a protected area without major urbanisation, while the second location was an area of intensive urbanisation. The highest concentrations of drug residues were determined in samples from the second sampling site, probably due to the anthropogenic interference. The study also observed the highest residual concentrations during the dry period.

The most significant concentrations of the monitored drug residues were found in samples from the rivers Cidlina (sampling site Luková), Výmola (sampling site Císařská Kuchyně), Chrudimka (sampling site Stan – a part of the village Vítanov) and in a sample from the Borecký stream (a stream with the sampling site in Souvrať). The residues of sulfamethoxazole, ibuprofen, naproxen and diclofenac were determined in a sample from the Cidlina (sampling site Luková).

Probably, the main source of contamination is the town Nový Bydžov with a population of 6 794 published in January 1, 2020 (MVCR 2020c). The wastewater from the hospital of Nový Bydžov is drained into the STP Nový Bydžov, which is emptied into the Cidlina river. Significant residual concentrations of sulfamethoxazole, naproxen and diclofenac were determined in a sample from the Výmola (sampling site Císařská Kuchyně). A significant source of contamination seems to be the town Úvaly with a population of 6 552 published in January 1, 2020 (MVCR 2020d). The wastewater from Polyclinic of Úvaly is diverted into the STP Úvaly, which is emptied into the Výmola river above the sampling site Císařská Kuchyně. Significant concentrations of ibuprofen, diclofenac and paracetamol (aceta-

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minophen) residues were determined in a sample from the Chrudimka river (sampling site Stan – a part of the village Vítanov). Probably, the main source of contamination is the town Hlinsko with a population of 9 584 published in January 1, 2020 (MVCR 2020a). The wastewater from the Hlinsko Polyclinic and several large industrial enterprises (the dairy producer Mlékárna Hlinsko, a.s.; Elektro-Praga Hlinsko a.s.) leads into the STP Hlinsko, which lies above the sampling site. The significant concentrations of ibuprofen and naproxen residues were determined in a sample from the Borecký stream (sampling site Souvrať). Probably, the main source of contamination is the village Mostek with a population of 1 177 published in January 1, 2020 (MVCR 2020b), although there is no health facility. However, the wastewater from Mostecké Lázně which leads into the STP Mostek may have caused the contamination of the Borecký stream.

Our results show that the most significant occurrence of the monitored drug residues are determined in small streams (with a low river flow rate) under large towns with a hospital or other health facilities. Also, the study by Liska et al. (2015) describes the occurrence of the highest concentrations of drug residues [including sulfamethoxazole, ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen)] mainly occurs in small streams with a small volume of water under medium and large urban settlements with a hospital or other health facilities.

Monitoring drug residues in the surface water and the natural ecosystem, in general, has been attracting increasing attention all around the world. The occurrence of residues of sulfonamides, non-steroidal anti-inflammatory drugs and analgesics-antipyretics is considered to be a worldwide problem, as evidenced by studies by a number of authors (Ferrer and Thurman 2012; Matongo et al. 2015; Lindim et al. 2016; Paiga et al. 2016; Bean et al. 2018; Rivera-Jaimes et al. 2018). The main reason is the potential toxicity, teratogenicity and genotoxicity of the drug residues and their mixtures on non-target organisms.

Antibiotic residues are observed due to the increasing bacterial resistance, which is currently one of the most significant human health risks (Liu et al. 2018; Qiao et al. 2018). Ben et al. (2019) mentioned a possible interaction of antibiotic residues with human intestine microbiome. This interaction may cause a microbial imbalance leading opportunistic intestine pathogens and other various intestine disorders (pseudomembranous colitis, colorectal carcinoma, etc.) to proliferate. Several cases of deaths caused by bacterial resistance and the subsequent incurability have been reported (Ben et al. 2019).

There are numerous studies based on the research of drug residue toxicity and its potential negative impact on the growth and development of non-target organisms, but knowledge of the chronic effects of these residues and the toxicity on aquatic organisms caused by their mixtures is still missing (Pomati et al. 2004; Sanderson et al. 2004; Gao et al. 2013).

In a study by Brain et al. (2004) dealing with the toxic impact of sulfonamides on *Lemna gibba,* sulfamethoxazole, sulfadimethoxine and sulfamethazine were found to be the most toxic sulfonamide substances. Sehonova et al. (2017) investigated the toxic effects of naproxen sodium and its mixture with tramadol hydrochloride during the subchronic exposure on the early life stages of *Cyprinus carpio*. The study confirmed a negative impact on the hatching, development, morphology, oxidative stress and fish mortality.

Due to the risks that the drug residues pose in an aquatic environment, further research is needed. In our opinion, the research should be focused on improving the cleaning capacity of STPs as a prevention of the negative effects on human health and non-target organisms. The aim of the further research should, therefore, be to minimise the risks to human health and non-target organisms.

The results of the present study confirmed the occurrence of significant concentrations of 5 residues out of the total 13 observed drugs – sulfamethoxazole, ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen). The highest concentration (1 600 ng/l) was determined for ibuprofen. In small low-flow streams, higher concentrations of drug residues were found due to the small dilution. A statistically significant negative correlation was detected between the river flow rate and the concentration of the residues in the case of ibuprofen, naproxen, diclofenac and paracetamol (acetaminophen).

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES

Bai Y, Meng W, Xu J, Zhang Y, Guo C. Occurrence, distribution and bioaccumulation of antibiotics in the Liao River Basin in China. Environ Sci: Process Impacts. 2014 Feb;16(3):586-93.

Bean TG, Rattner BA, Lazarus RS, Day DD, Burket SR, Brooks BW, Haddad SP, Bowerman WW. Pharmaceuticals in water, fish and osprey nestlings in Delaware River and Bay. Environ Pollut. 2018 Jan;232:533-45.

Ben Y, Fu C, Hu M, Liu L, Wong MH, Zheng C. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environ Res. 2019 Feb;169:483-93.

Brain RA, Johnson DJ, Richards SM, Sanderson H, Sibley PK, Solomon KR. Effects of 25 pharmaceutical compounds to Lemna gibba using a seven-day static renewal test. Environ Toxicol Chem. 2004 Feb;23(2):371-82.

Castiglioni S, Zuccato E. Occurrence of illicit drugs in wastewater and surface water in Italy. In: Castiglioni S, Zuccato E, Fanelli R, editors. Illicit drugs in the environment. Milan, Italy: Willey; 2011. p. 137-51.

Ferrer I, Thurman EM. Analysis of 100 pharmaceuticals and their degradates in water samples by liquid chromatography/quadrupole time-of-flight mass spectrometry. J Chromatogr A. 2012 Oct 12;1259:148-57.

Forejtova S. Systemova nesteroidni antirevmatika [Systemic non-steroidal anti-rheumatic drugs]. In: Pavelka K, Vencovsky J, Senolt L, Horak P, Olejarova M, Tomcik M, Zavada J, Stepan J, editors. Farmakoterapie revmatickych onemocneni [Pharmacotherapy of rheumatic diseases]. Praha, Czech Republic: Maxdorf; 2017. p. 33-46.

Gao L, Shi LJ, Yuan T. Growth inhibitive effect of typical antibiotics and their mixtures on Selenastrum capricornutum. J Environ Health. 2013;30(6):475-8.

Gerrett D. Pharmacology. In: Turner W, Merriman L, editors. Clinical skills in treating the foot. London, UK: Churchill Livingstone; 2005. p. 161-90.

Hamscher G, Powelzick HT, Hoper H, Nau H. Antibiotics in soil: Routes of entry environmental concentrations, fate and possible effects. In: Kummerer K, editor. Pharmaceuticals in the environment: Sources, fate, effects and risks. Berlin, Germany: Springer International Publishing; 2004. p. 139-47.

Hamscher G, Powelzick HT, Sczesny S, Nau H, Hartung J. Antibiotics in dust originating from a pig-fattening farm: A new source of health hazard for farmers? Environ Health Perspect. 2003 Oct;111(13):1590-4.

Hirsch R, Ternes T, Haberer K, Kratz KL. Occurrence of antibiotics in the aquatic environment. Sci Total Environ. 1999 Jan 12;225(1-2):109-18.

Hruska K, Franek M. Sulfonamides in the environment: A review and a case report. Vet Med-Czech. 2012 Jan; 57(1):1-35.

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 2008 Jul;42(13):3498-518.

Kolpin DW, Skopec M, Meyer MT, Furlong ET, Zaugg SD. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Sci Total Environ. 2004 Jul 26; 328(1-3):119-30.

Lindim C, Van Gils J, Georgieva D, Miekenyan O, Cousins IT. Evaluation of human pharmaceutical emissions and concentrations in Swedish river basins. Sci Total Environ. 2016 Dec 1;572:508-19.

Liska M, Soukupova K, Kule L, Metelkova A, Kozeluh M. Vyskyt farmak v povrchovych a odpadnich vodach povodi Vltavy „ve svetle" konference Water and Health – Zeneva/Annemasse 2015 [Occurrence of pharmaceuticals in surface water and wastewater of the Vltava river basin „in light of" the Water and Health Conference – Geneva/Annemasse 2015]. Vodní hospodářství. 2015;11:1-5. Czech.

Liu L, Wu W, Zhang J, Lv P, Xu L, Yan Y. Progress of research on the toxicology of antibiotic pollution in aquatic organisms. Acta Ecolog Sinica. 2018 Feb;38(1):36-41.

Marsik P, Rezek J, Zidkova M, Kramulova B, Tauchen J, Vanek T. Non-steroidal anti-inflammatory drugs in the watercourses of Elbe basin in Czech Republic. Chemosphere. 2017 Mar;171:97-105.

Martinkova J, Grim J, Hojdikova H, Chladek J, Chladkova J, Kulda K, Libiger J. Farmakologie pro studenty zdravotnickych oboru [Pharmacology for students of medical fields]. Praha, Czech Republic: Grada Publishing; 2018. 520 p. Czech.

Matongo S, Birungi G, Moodley B, Ndungu P. Pharmaceutical residues in water and sediment of Msunduzi river, KwaZulu-Natal, South Africa. Chemosphere. 2015 Sep; 134:133-40.

MVCR. Pocet obyvatel Hlinsko [Number of inhabitants Hlinsko] [Internet]. 2020a [cited 2020 Jul 15]. Available from: https://www.mistopisy.cz/pruvodce/obec/10560/novy-bydzov/pocet-obyvatel/. Czech.

MVCR. Pocet obyvatel Mostek [Number of inhabitants Mostek] [Internet]. 2020b [cited 2020 Jul 15]. Available from: https://www.mistopisy.cz/pruvodce/obec/10560/novy-bydzov/pocet-obyvatel/. Czech.

MVCR. Pocet obyvatel Novy Bydzov [Number of inhabitants Novy Bydzov] [Internet]. 2020c [cited 2020 Jul 15]. Available from: https://www.mistopisy.cz/pruvodce/obec/10560/novy-bydzov/pocet-obyvatel/. Czech.

https://doi.org/10.17221/180/2020-VETMED

MVCR. Pocet obyvatel Uvaly [Number of inhabitants Uvaly] [Internet]. 2020d [cited 2020 Jul 15]. Available from: https://www.mistopisy.cz/pruvodce/obec/10560/novy-bydzov/pocet-obyvatel/. Czech.

Nikolaou A, Meric S, Fatta D. Occurrence patterns of pharmaceuticals in water and wastewater environments. Anal Bioanal Chem. 2007 Feb;387(4):1225-34.

Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJI, Arshad M, Mahmood S, Ali A, Khan AA. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature. 2004 Feb 12;427(6975):630-3.

Olejarova M. Revmatologie v kostce [Rheumatology in a nutshell]. Praha, Czech Republic: Triton; 2008. 231 p. Czech.

Paiga P, Santos LHMLM, Ramos S, Jorge S, Silva JG, Delerue-Matos C. Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Sci Total Environ. 2016 Dec 15;573:164-77.

Pavelka K. Nesteroidni antirevmatika [Non-steroidal antirheumatics]. In: Klener P, editor. Vnitrni lekarstvi, Svazek VII, Revmatologie [Internal Medicine, Volume VII, Rheumatology]. Praha, Czech Republic: Galén; 2002. p. 134-7.

Pomati F, Netting AG, Calamari D, Neilan BA. Effects of erythromycin, tetracycline and ibuprofen on the growth of Synechocystis sp. and Lemna minor. Aquat Toxicol. 2004 May 12;67(4):387-96.

Qiao M, Ying GG, Singer AC, Zhu YG. Review of antibiotic resistance in China and its environment. Environ Int. 2018 Jan;110:160-72.

Rivera-Jaimes JA, Postigo C, Melgoza-Aleman RM, Acena J, Barcelo D, Lopez de Alda M. Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: Occurrence and environmental risk assessment. Sci Total Environ. 2018 Feb 1;613-614:1263-74.

Rogers IH, Birtwell IK, Kruzynski GM. Organic extractables in municipal wastewater Vancouver, British Columbia. Water Qual Res J Can. 1986 May;21(2):187-204.

Sanderson H, Brain RA, Johnson DJ, Wilson CJ, Solomon KR. Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular and sex hormones. Toxicology. 2004 Oct 15; 203(1-3):27-40.

Sehonova P, Plhalova L, Blahova J, Doubkova V, Prokes M, Tichy F, Fiorino E, Faggio C, Svobodova Z. Toxicity of naproxen sodium and its mixture with tramadol hydrochloride on fish early life stages. Chemosphere. 2017 Dec;188:414-23.

Stancova V, Zikova A, Svobodova Z, Kloas W. Effects of the non-steroidal anti-inflammatory drug (NSAID) naproxen on gene expression of antioxidant enzymes in zebrafish (Danio rerio). Environ Toxicol Pharmacol. 2015 Sep;40(2):343-8.

Sukul P, Spiteller M. Sulfonamides in the environment as veterinary drugs. In: Ware GW, Nigg HN, Doerge DR, editors. Reviews of environmental contamination and toxicology, vol 187. New York, USA: Springer; 2006. p. 67-101.

Swan GE, Cuthbert R, Quevedo M, Green RE, Pain DJ, Bartels P, Cunningham AA, Duncan N, Meharg AA, Oaks JL, Parry-Jones J, Shultz S, Taggart MA, Verdoorn G, Wolter K. Toxicity of diclofenac to Gyps vultures. Biol Lett. 2006 Jun-22;2(2):279-82.

Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 1998 Nov;32(11): 3245-60.

Veras TB, De Paiva ALR, Duarte MMMB, Napoleao DC, Cabral JJdSP. Analysis of the presence of anti-inflammatories drugs in surface water: A case study in Beberibe river – PE, Brazil. Chemosphere. 2019 May;222:961-9.

Watts CD, Crathorne B, Fielding M, Killops SD. Nonvolatile organic compounds in treated waters. Environ Health Perspect. 1982 Dec;46:87-99.

Zhou JL, Zhang ZL, Banks E, Grover D, Jiang JQ. Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. J Hazard. Mater. 2009 Jul 30;166(2-3):655-61.

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