PHARMACEUTICALS AND PERSONAL CARE PRODUCTS ARE EMERGING ENVIRONMENTAL CONTAMINANTS ALSO IN DEVELOPING COUNTRIES – ENANTIOSELECTIVE ANALYSIS OF THEIR TRANSFORMATION

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Introduction

Pharmaceuticals and personal care products (PPCPs) have been detected in many environmental samples taken in highly industrialized countries in wastewater and surface waters, and they are thus classified as emerging pollutants[1-4]. However, at the beginning of the present study, it was generally assumed that in developing countries the "classical" pesticides of the first and second generation are prevailing in all environmental compartments. As will be shown herein, this assumption has to be modified considerably.

An additional important aspect comprises the chirality of many pharmaceutically active compounds, because the stereoisomers may trigger different biological effects. For example, the chiral naproxen is administered in the pure *S*-form due to the fact that the antipode shows toxic effects[5]. Newer results revealed that also the enantiomers of pharmaceuticals like propranolol or fluoxetine have different toxic effects[6,7]. In the recent years, emphasis was placed on the analysis of single enantiomers also in the environment in order to get a more detailed insight into the enantiomeric composition and the changes of chiral compounds. Thus it could be revealed that compounds like ibuprofen and naproxen undergo changes in the composition of their respective enantiomers during sewage treatment plant passage[8,9]. The extremely high concentrations of the parent compounds encountered in the present Pakistanian water samples allowed a detailed investigation of the formation of new transformation products as well as of the enantioselectivity of the respective transformation process.

Materials and methods

In Karachi, Pakistan, surface water samples were collected during two campaigns in December 2006 and April 2007 (Fig. 1). Sample 1 was taken from the Malir River, while samples 7 and 9 stem from the Lyari River, the two major rivers flowing through Karachi. Sample 3 was taken in the mangrove lagoon, which is part of Karachi harbor receiving effluents from the center parts of the city. Samples 4, 5, 6 and 8 were taken from an open drainage canal system (Korangi drain) receiving untreated residential and industrial effluents as well as wash-off and rain water from the Landhi residential district. Sample 2 was taken from the end of a pipe eluting waste waters from the district of Clifton across the beach into the Arabian Sea.

All samples were taken in 2.5 L amber glass bottles with a sampling device designed in the working group and filtered through a glass fibre filter (GF-A, Whatman), a sample volume of 2 L of which was extracted over 1 g of Oasis HLB (Waters, Germany). After extraction of the sample the solid phase was dried with nitrogen, before being eluted with 40 mL of methanol. The resulting methanol eluates were then evaporated to dryness and derivatized with methyl chloromethanoate (MCM), according to ref[10], to form the methyl esters (COOH-functions) or carbonate diesters (HO-functions), respectively. The resulting *n*-hexane phase of the derivatization reaction was spiked with 100 µL of an internal standard (mecoprop methyl ester [1 µg/mL]) and evaporated under a gentle stream of nitrogen to a final volume of 100 µL. GC-MS analysis was performed on a Magnum ITD 40 (Finnigan MAT, Bremen, Germany) ion trap mass spectrometer with the following conditions: EI at 70 eV, manifold temperature 473 K, emission current 10 µA, dwell time 100 µs, and scan range 40-500 m/z (full scan mode). It was coupled to a Varian 3400 GC system (Sunnyvale, CA, USA), separation was performed on a VF-5MS column, analogue to DB-5 (Varian, Sunnyvale, CA, USA), length 30 m, ID 0.2 mm, film thickness 0.33 µm, carrier gas Helium 5.0, transfer line 523 K run with an A 200 SE autosampler (CTC Analytics, Zwingen, Switzerland), injected volume 2 µL. The column was temperature programmed as follows: 333 K (2.5 min) with 6 K/min to 523 K (kept for 15 min). For the enantioselective separation a modified β-Cyclodextrin

was used (2.3-di-*O*-methyl-6-*tert*-butyl-dimethylsilyl-*β*-cyclodextrin [Hydrodex-*ß*-6TBDM] from Macherey-Nagel, Germany, length 25 m, ID 0.25 mm, film thickness 0.1 µm). The column was temperature programmed as follows: 343 K (15 min) with 2 K/min to 493 K (kept for 30 min) with a carrier gas pressure of 12 PSI.

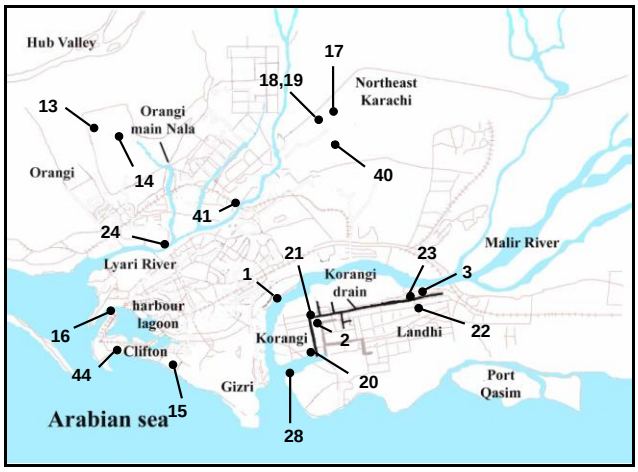

Fig. 1. Map of Karachi showing the rivers and the draining system, sampling locations are marked (X)

Results and discussion

In the present investigation an integrative approach was followed combining chemical-analytical and ecotoxicological aspects. 768 fractions from 32 samples were screened for their ecotoxicological relevance by the luminescent bacteria test. The samples originate from all parts of the drinking water circuit, including the sources (ground water and surface water), distribution, processing and tapping points as well as waste water. The determined pollution status indicates the alarming condition of the drinking water circuit. However, contrary to our expectation, not the "classical" pesticides of the first and second generation were prevailing. The waste and surface waters of the city area of Karachi were severely contaminated with PPCPs and industrial chemicals. As an example, in Figure 2 the impact by mefenaminic acid, ibuprofen, diclofenac and naproxen is shown. for the respective sampling stations, while in Figure 3 the concentrations of some pesticides such as MCPA, 2,4-D, atrazine and chlorpyrifos are displayed. Please note the different scales of PPCP and pesticide concentrations.

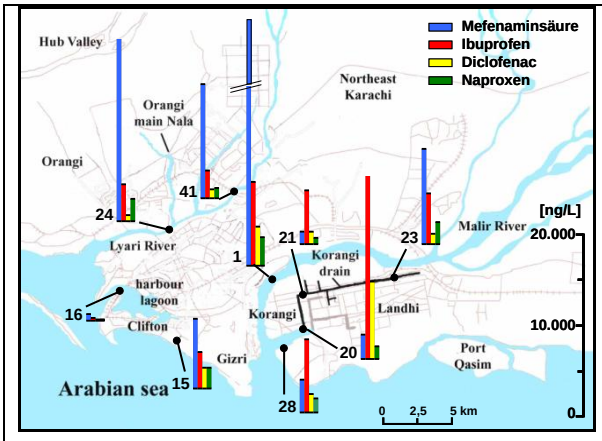

Fig. 2: Distribution of mefenaminic acid, ibuprofen, diclofenanc and naproxen in Karachi water samples.

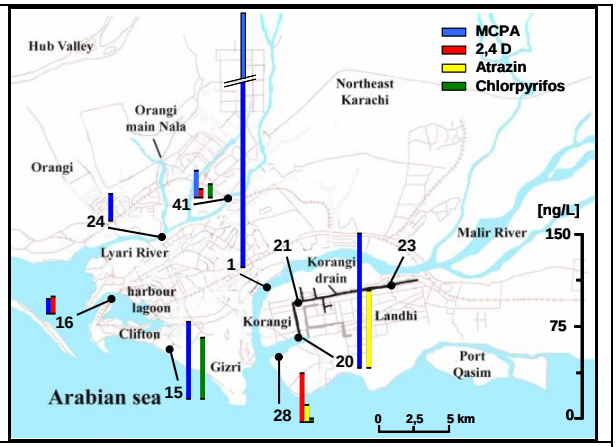

Fig. 3: Distribution of MCPA, 2,4-D, atrazine and chlorpyrifos in Karachi water samples

Notable additional contamination that refers to classical pesticides was determined for endosulfane-lactone, a transformation product of endosulfane, and particularly for some isocyanates, transformation products of phenyl urea pesticides. This means that the parent compounds of classical pesticides do not any longer play a major role,

however, some of their transformation products were still found as contaminants in the drinking water of Karachi as summarized in Figure 4

As an example for industrial chemicals the concentrations of bromobenzene, 1-aminoanthrachinone and of chloroanilines are given in Figure 5 for surface and waste water stations, while in Figure 6 the consequences of the bromobenzene load for the drinking water of the city of Karachi is shown. PPCP concentrations in Karachi drinking water may achieve maximum values of up to 400 ng/L while industrial chemical concentrations may attain maximum values of about 7000 ng/L.

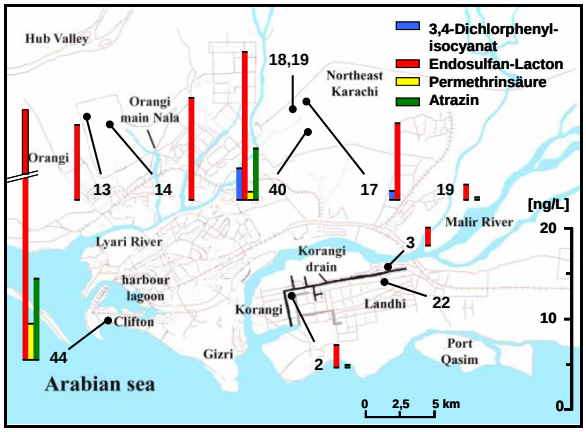

Fig. 4: Distribution of 3,4-dichlorophenylisocyanate, endosulfane-lactone, permethrin acid und atrazine in Karachi drinking water samples

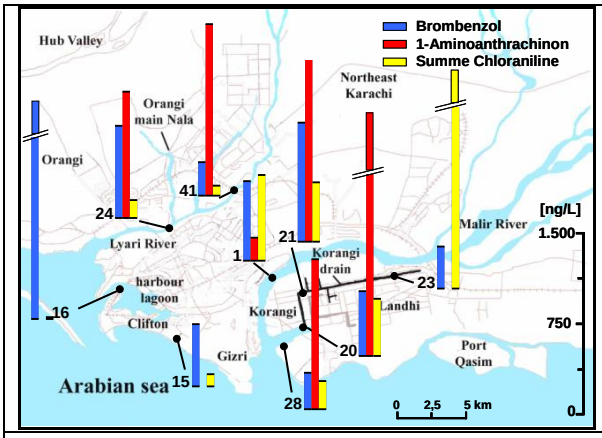

Fig. 5: Distribution of bromobenzene, 1-amino-anthrachinone and the sum of chloroanilines in Karachi surface water samples

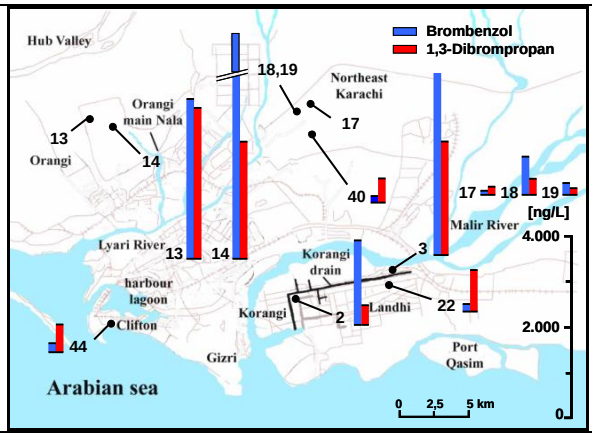

Fig. 6: Distribution of bromobenzene and 1,3-dibromopropane in Karachi drinking water samples.

The high concentrations of the parent compounds and their transformation products allowed the determination of the molecular structure of several "new" environmental metabolites. As mefenaminic acid was found in all environmental water samples in Karachi, the respective transformation product 3-hydroxy-mefenaminic acid was ubiquitously identified and verified by GC/MS (Figure 7). Please note that the differences between the mass spectra shown in 7a and 7b are due to the derivatization of our sample compound by methyl chloromethanoate.

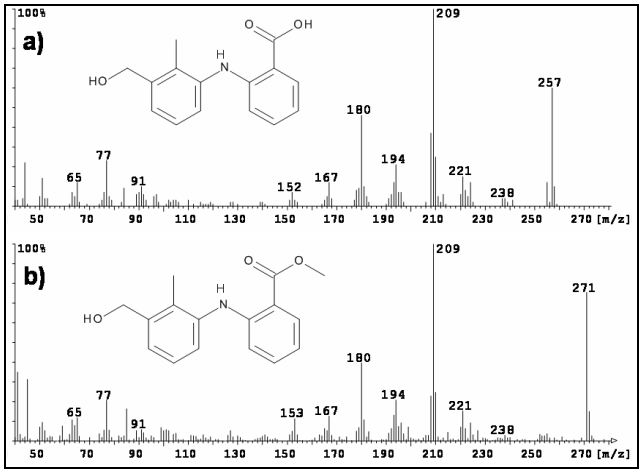

Fig. 7: Mass spectra of 3-hydroxy-mefenaminic acid; a.) reference spectrum; b.) from sample station #1.

Furthermore, the high concentrations of chiral environmental pollutants allowed the determination of the stereoselectivity of the respective biotic transformation processes. An example is given in Figs. 8a and 8b for ibuprofen and it main transformation product hydroxy-ibuprofen.

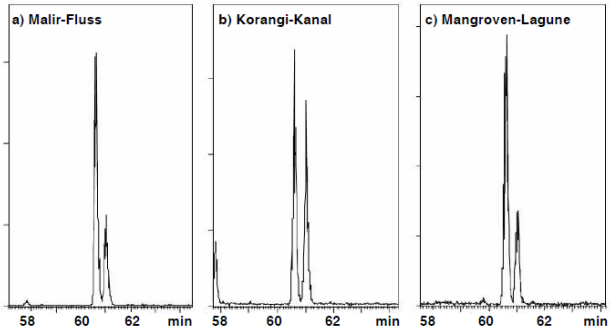

Fig. 8a) Ibuprofen enantiomers in environmental samples of Karachi

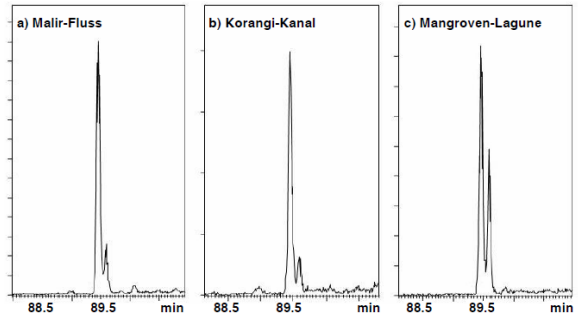

Fig. 8b) Hydroxy-ibuprofen enantiomers in environmental samples of Karachi

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References

1. Ternes TA, Stumpf M, Schuppert B, Haberer K (1998), *Vom Wasser* 90: 295
2. Metcalfe CD, Koenig BG, Bennie DT, Servos M, Ternes TA, Hirsch T (2003), *Environ Toxicol Chem.* 22: 2872
3. Ternes TA (2001), *Tr. Anal. Chem.* 20: 419
4. Khetan SK, T.J. Collins TJ (2007), *Chem Rev* 107: 2319
5. Roszkowski AP, Rooks WH, Tomolouis AJ, Miller LM (1971), *J Pharmacol Exp Ther* 179: 114
6. Stanley JK, Ramirez AJ, Mottaleb M, Chambliss CK, Brooks BW (2006), *Environ Toxicol Chem* 25: 1780
7. Stanley JK, Ramirez AJ, Chambliss CK, Brooks BW (2007), *Chemosphere* 69: 9
8. Buser HR, Poiger T, Müller MD (1999), *Environ Sci Techno.* 33 : 2529
9. Matamoros V, Hijosa M, Bayona JM (2009), *Chemosphere* 75: 200
10. Weigel S, Kallenborn R, Hühnerfuss H (2004); *J. Chromatogr. A* 1023: 183