QUALITATIVE NON-TARGET SCREENING OF TRACE ORGANICS IN GREYWATER TREATED IN VERTICAL-FLOW CONSTRUCTED WETLANDS

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ABSTRACT

Effluent and influent of a constructed wetlands treating separated greywater of an eco-settlement were subdued to liquid/liquid extraction with dichloromethane as well as to solid phase extraction. Extracts were analysed by GC/MS and the detected organic compounds tentatively identified. Major compounds detected in the solid phase extracts of the effluent were di-*tert.*-butylphenol, *tert.*-butylmethoxyphenol ("butylated hydroxyanisol", BHA), two unidentified phthalates, di-*tert.*-butyloxa-*spiro*-decadienedione, acetophenon, di-*tert.*-butylbenzo-quinone, an *iso*-alkane, a squalene isomer, hexadecanoic acid, tris-chloropropyl phosphate, an acephylline derivative and an unidentified nitrogenous heterocycle, while only two unidentified phthalates represented major peaks in liquid extracts. The minor organics in the solid phase extracts were methylbenzotriazol, benzoquinoline, and two alkanes, while in the liquid extract a not further characterised alkane, a squalene isomer, diethyleneglycol, three different polyethylene glycols, hexadecanoic acid, benzylbutyl phthalate, another unidentified phthalate, ethylhexanoic acid hexadecyl ester, triethyl citrate, tris-chloropropyl phosphate, methylbenzotriazol, and 1*H*-benzotriazol were minor constituents. In the original greywater a higher number of particular organics have been detected, predominantly long chain aliphatic carboxylic acids. It is of concern that a persistent flame retardant (tris-chloropropyl phosphate) and a couple of phthalates as well as BHA, an endocrine disrupting chemical, were tentatively identified in biologically treated greywater. This has to be considered when biologically treated greywater will be reused for high quality demand. Most of the detected trace organics were related to household chemicals, personal care products and foodstuffs.

KEYWORDS: Constructed wetlands, ecological sanitation, greywater, trace organics

INTRODUCTION

Greywater as the predominant stream of domestic wastewater is believed to be rather lowly contaminated with nutrients and inorganic as well as organic hazardous substances. Therefore, it is looked at as a good water resource for reuse after proper treatment by many researchers. Greywater reuse e.g. for gardening, car-washing, laundry, or toilet flushing represents the largest potential source of water savings in domestic residence. Although Jefferson et al. (2004) have stated with regard to greywater that "high COD/BOD ratio coupled with a nutrient and micro metal imbalance suggest biological processes may encounter performance and operational difficulties", aerobic biological processes turned out to be suitable for greywater treatment: membrane bioreactors (Lesjean and Gnirss 2006), sequencing batch reactors (Lamine et al. 2007), and constructed wetlands (Gross et al. 2006, Li et al. 2003) have been investigated for greywater treatment.

In the eco-settlement Luebeck/Flintenbreite, Germany, a semi-centralised sanitation concept is operated in a peri-urban area since 2000. Biological treatment of separately collected greywater in vertical intermittently operated constructed wetlands did not lead to any operational problems and yields rather low TOC effluent concentrations (5 to 15 mg/l) (Li et al. 2003). Organics characterised by size exclusion chromatography with organic carbon detection in the effluent exhibiting a non-purgeable TOC concentration of 6 mg/l were polysaccharides, humic substances, building blocks (subunits of humic substances) and only to a very low extent "amphiphilic and neutral organics" which represent low-molecular weight trace organics (Gulyas et al. 2005). But as Eriksson et al. (2003) detected many particular organics and Palmquist and Hanaeus (2005) found hazardous organics like flame-retardants, nonylphenol, octylphenol and organotin compounds in the µg/l range in untreated greywater, this preliminary study was performed in order to clarify which trace organics can be detected in greywater subsequent to biological treatment.

MATERIALS AND METHODS

Greywater treatment and sampling. At present, 103 inhabitants are connected to the semi-central sanitation system at the eco-settlement Luebeck/Flintenbreite. Greywater and blackwater are collected and treated separately. The greywater is drained by gravity and treated in a 280 m^2 subsurface vertical flow constructed wetlands (fig. 1) with intermittent feeding (ensuring aerobic conditions) from a septic tank about 35 times a week (depending on filling level of septic tank) for 13.25 min by means of a submerged pump.

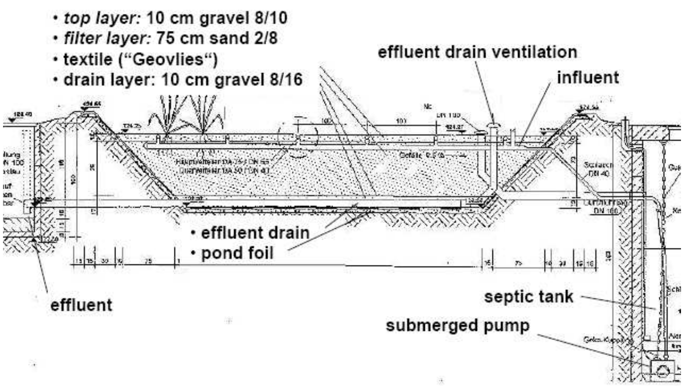

Figure 1 – Cross-sectional drawing of the intermittently operated subsurface vertical flow constructed wetlands for greywater treatment at the eco-settlement Luebeck/Flintenbreite, Germany

An influent grab sample was collected from the septic tank and an effluent sample directly from the effluent tube and shipped in tightly sealed Duran glass bottles to the laboratory.

Extraction of trace organics. For solid phase extraction (SPE), abselut NEXUS cartridges (60 mg, Varian) were applied without pre-conditioning. Greywater samples (700 ml constructed wetlands effluent or 100 ml influent) were sucked through the cartridges by vacuum. Subsequently the cartridges were rinsed with 5 ml of deionised water and dried by sucking air through the cartridges for at least 30 min. Finally, the organics were eluted with 1 ml methanol (analytical grade). For liquid/liquid extraction (LLE), 220 ml of each sample were extracted three times with 10 ml dichloromethane (analytical grade). The combined extracts were dried with Na_2SO_4 and concentrated to 1 ml by vacuum evaporation.

Analyses. Extracts were analysed with a gas chromatograph Agilent 6890N equipped with a mass selective detector 5975B; injector: Gerstel KAS 4, 300°C; column: HP-5ms, 30 m, I.D. 0.25 mm, film thickness 0.25 µm; carrier gas: He, 1 ml/min; temperature program: 70°C (2 min), 8°C/min, 290°C (15 min); ionisation: EI 70 eV, MS source 230°C. Mass spectra were compared to a computerised spectra library NIST 05a.

RESULTS AND DISCUSSION

Figure 2 shows total ion current gas chromatograms of solid phase and liquid extracts of the influent and the effluent of the constructed wetlands indicating slight differences in extraction selectivities of SPE and LLE. In the influent sample more than 70 organic compounds were tentatively identified or at least characterised by GC/MS following LLE and about 40 following SPE. This refers probably to the higher volume (220 ml) subdued to LLE than to SPE (100 ml influent). For effluent sample preparation conversely a higher volume (700 ml) was prepared by SPE than by LLE (220 ml). Some of the organics found with the two extraction procedures were identical, some were not due to differences in selectivity of the two extraction methods. The fact that different types of extraction methodologies are complementing one another might explain that Eriksson et al. (2003) have tentatively identified more organics in non-treated greywater (although not containing kitchen wastewater and washing machine effluent) than found in this study, because they applied four different solid phases for SPE in their study.

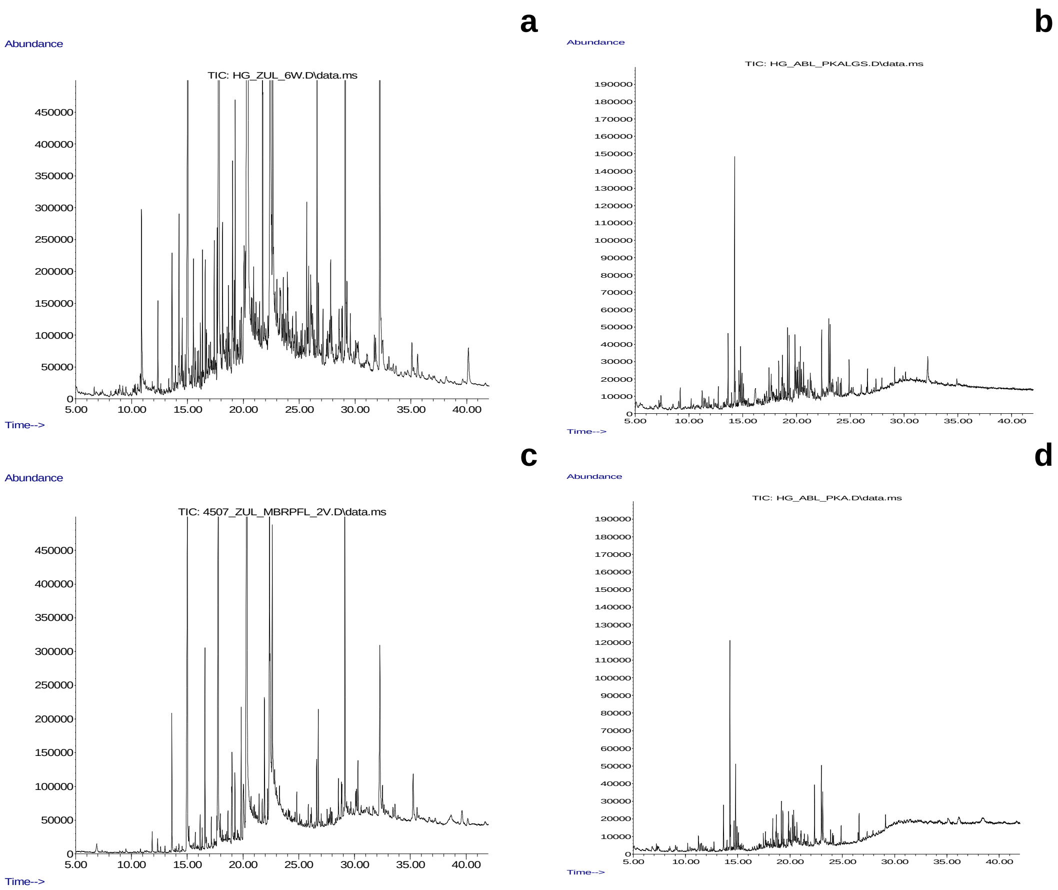

Figure 2 – Total ion current chromatograms of constructed wetlands influent (a: SPE; c: LLE, diluted 1:20 with dichloromethane) and effluent (b: SPE; d LLE)

Identification of the particular organics by their mass spectra was tentative, i.e. the results were not verified by re-analysing reference solutions of the pure compounds. With such a method being much more expensive and time-consuming more certitude of identification would be obtained. Nevertheless, the identification results displayed in table 1 are plausible, because many of the identified substances are contained in materials like household chemicals, personal care products and foodstuffs which partially end up in greywater. Moreover, many of the substances contained in table 1 have also been identified in greywater by Eriksson et al. (2003). Table 1 contains all 28 organics tentatively identified (or at least characterised e.g. as "phthalate") in the constructed wetlands effluent (shaded fields). Additionally, a couple of organics of interest found in the untreated greywater are displayed, but not all. It seems to be confusing that some organics were found in the effluent but not in the influent. This can be explained by two reasons: Firstly, the two samples were taken nearly at the same time, i.e. they were no corresponding samples. Secondly, substances being present in the influent in very small concentrations might be hidden by highly concentrated constituents co-eluted at the same retention time. Thus, the mass spectrum of the trace compound might not carry weight leading to lack of detection. An example are hexadecanoic acid and a not further characterised phthalic acid ester, both exhibiting similar retention times (20.3 min): Hexadecanoic acid represented the main peak in the influent LLE sample, but no phthalate referring to this retention time was detected in the influent, while in the effluent LLE sample both substances were detected with the respective phthalate representing the main peak and hexadecanoic acid being only a minor component in that chromatogram due to biodegradation.

Table 1 gives also some semiquantitative information reflecting the ratios of the peak areas to the area of the largest peak. Comparison to quantitative GC/MS analyses with municipal effluents (Gulyas 1997) suggests that the concentrations of organis detected in the constructed wetlands effluent by GC/MS are in the µg/l range.

Table 1 – Organics tentatively identified (or at least characterised) in greywater after and before treatment in constructed wetlands

Substance [a)]	**semiquantitative characterisation** [b)]		**Possible source or use**
	effluent	**influent**	
Hydrocarbons			
Iso-alkane (7.21)	trace (SPE)	n.d.	Many skin care products (e.g. baby oils) contain mineral instead of natural oils
Alkane (11.24)	minor (SPE)	n.d.	"
Iso-alkane (14.64)	major (SPE)	n.d.	"
Alkane (25.08)	trace (LLE)	n.d.	"
Squalene isomer [c, e)] (29.13)	major (SPE), minor (LLE)	major (SPE, LLE)	food additive; skin care products
Squalene isomers (28.87, 28.91)	n.d.	traces (LLE)	"
Alkene (15.17)	n.d.	minor (LLE)	
Decene [c)]	n.d.	trace (SPE), minor (LLE)	
Octadecene [c, e)]	n.d.	minor (SPE)	fragrances, flavors, personal care products (e.g. shaving creme)
Alcohols/ethers			
Diethyleneglycol	trace (LLE)	n.d.	illegally used as counterfeit glycerol and sold internationally as a component of cough syrup and toothpaste
Polyethyleneglycol (15.07)	minor (LLE)	n.d.	skin cleaners, oven cleaners
Polyethyleneglycol (18.84)	minor (LLE)	n.d.	"
Polyethyleneglycol (22.16)	trace (LLE)	n.d.	"
Cholestenol (cholesterol) [c)]	trace (SPE)	minor (SPE)	foodstuffs like meat, egg yolk
Sitosterol [c)]	n.d.	trace (SPE)	vegetable foodstuffs like wheat germ, corn oils, soybeans
Benzyl alcohol [e)]	n.d.	trace (LLE)	many essential oils and foodstuffs
Phenylethanol [e)]	n.d.	trace (LLE)	main component of rose oils; flavouring substance for food
Phenoxyethanol [c)]	n.d.	trace (SPE, LLE)	preservative in skincare products
Menthol [c)]	n.d.	trace (LLE)	oral hygiene products, lip balms, antipruritics, decongestants for chest and sinuses

a) numbers in brackets give retention times for substances which were not identified, but only characterised
b) n.d.: not detectable; ratio of peak area to area of largest peak ("main") in the respective chromatogram below 5 %: "trace"; 5 to 20 %: "minor"; 20 to 100 %: "major"
c) also found in untreated greywater by Eriksson et al. (2003)
d) also found in untreated greywater by Palmquist and Hanaeus (2005)
e) also found in domestic laundry wastewater by Seo et al. (2005)

Table 1, continued

Substance [a]	semiquantitative characterisation [b] effluent	influent	Possible source or use
Phenols			
Tert.-butylmethoxy-phenol (BHA)	major (SPE)	n.d.	antioxidant in creams, ointments and cosmetics like e.g. lipsticks; also in foodstuffs like instant soups, almond paste, salty snacks, baking mixtures
Di-*tert.*-butylphenol	**main (SPE)**	minor (SPE)	antioxidant in mineral oils
Other di-*tert.*-butyl-phenol isomer	n.d.	trace (SPE)	
Phenol	n.d.	trace (LLE)	urine
Methylphenol (cresol)	n.d.	trace (LLE)	disinfectant; flavouring substance in foodstuffs; liquors like Scotch Whiskey
Propylphenol	n.d.	trace (SPE, LLE)	
Alkylphenol (10.69)	n.d.	trace (LLE)	
Propylparaben	n.d.	trace (SPE)	preservative in creams and lotions, also found in many plants
Aldehydes/ketones			
Acetophenon	major (SPE)	n.d.	fragrance (naturally occurring component of a large number of foodstuffs and essential oils)
Di-*tert.*-butyl-oxa-*spiro*-decadienedione	major (SPE)	n.d.	fragrance oxidation product?
Di-*tert.*-butyl-benzoquinone	major (SPE)	n.d.	contaminant of the antioxidant *tert.*-butylhydroxyquinone (TBHQ).
Methylbenzaldehyde	n.d.	trace (LLE)	flavor compositions for cherry, coconut, nut, berry fruits and floral-sweet compounds
Nonanal	n.d.	trace (LLE)	fragrance (e.g. in citrus and rose oils)
Decanal	n.d.	trace (SPE)	fragrance (component of e.g. neroli and citrus peel oil)
Carboxylic acids			
Hexadecanoic acid (palmitic acid) [c]	major (SPE), minor (LLE)	**main (SPE, LLE)**	emulsifier for facial creams and lotions, shaving cream formulations
Octanoic acid [c]	n.d.	trace (LLE)	
Decanoic acid	n.d.	trace (LLE)	
Dodecanoic acid (laurylic acid) [c]	n.d.	major (SPE, LLE)	
Methyldodecanoic acid	n.d.	trace (LLE)	
Tetradecanoic acid (myristic acid) [c]	n.d.	major (SPE, LLE)	
Pentadecanoic acid	n.d.	minor (SPE), trace (LLE)	
Heptadecanoic acid	n.d.	minor (SPE), trace (LLE)	
Octadecanoic acid (stearic acid) [c]	n.d.	major (SPE, LLE)	emulsifier, thickener; Ca and Zn salts are used as anti-caking agents.
Hexadecenoic acid [c]	n.d.	minor (SPE, LLE)	
Octadecenoic acid [c]	n.d.	major (SPE, LLE)	
Octdecadienoic acid	n.d.	minor (LLE)	
Carboxylic acid esters			
Phthalate (19.19)	minor (LLE)	trace (SPE)	Phthalates are used to enhance fragrances and are present in household dust.
Phthalate (20.35)	major (SPE), **main (LLE)**	n.d.	"

Phthalate (26.61)	major (SPE, LLE)	minor (SPE), major (LLE)	"

Table 1, continued

Substance [a)]	semiquantitative characterisation [b)] effluent	influent	Possible source or use
Carboxylic acid esters			
Benzylbutylphthalate [d)]	trace (LLE)	trace (SPE)	plasticizer in PVC floors; found in PVC floor wipes
Ethylhexanoic acid hexadecyl ester	trace (LLE)	trace (SPE)	emollient (ethylhexyl hexadecanoate)
Triethyl citrate	trace (LLE)	trace (LLE)	food additive (flavoring agent, solvent vehicle, surface-active agent)
Several esters of C_{16} and C_{18} saturated and unsaturated acids	n.d.	traces	Many long chain fatty acid long chain alcohol esters are used as emollients in skin care products.
2-Ethylhexyl-*trans*-4-methoxycinnamate	n.d.	trace (LLE)	UV light absorber in sunscreens
2*H*-1-Benzopyranone (coumarin)	n.d.	trace (LLE)	fragrance
Phosphoric acid ester			
Tris-(1-chloropropyl)-phosphate	major (SPE), trace (LLE)	n.d.	flame retardant
Nitrogenous organics			
Acephylline derivative	major (SPE)	n.d.	cough syrup; acephylline piperazine for asthma treatment
Nitrogenous heterocycle (16.15)	major (SPE)	n.d.	
Benzoquinoline	minor (SPE)	n.d.	grilling of chicken meat
Methylbenzotriazol	minor (SPE), trace (LLE)	n.d.	silver protection component in dishwashing agents
1*H*-Benzotriazol	trace (LLE)	n.d.	"
Indole	n.d.	trace	artificial and natural jasmin oil, other exotic floral oils, coffee
3-Methyl-1*H*-indole (skatole)	n.d.	trace	feces, essential oils like jasmin oil, fragrance and fixative in many perfumes
1.3-Dihydro-1*H*-indol-2-one	n.d.	trace (LLE)	
Hexahydro-methano-1*H*-indole	n.d.	trace (LLE)	
Caffeine	n.d.	trace	coffee
Methylpyrrolidinyl-pyridine (nicotine)	n.d.	trace	Nicotine is contained in tobacco and in lower quantities also in tomato, potato, eggplant and green pepper.
2-Methylthiobenzo-thiazol	n.d.	trace (SPE)	
N,N-Dimethyl-1-decanamine	n.d.	minor (SPE)	
EDTA	n.d.	trace	complexing agent in many personal care products

In original greywater extracted by SPE, the predominant organics tentatively identified were carboxylic acids (dodecanoic to octadecanoic acid) and squalene. In lower concentrations, 2-phenoxyethanol, propylphenol, di-*tert.*-butylphenol, several not further characterised phthalates, propylparaben, cholestenol, sitosterol, EDTA, caffeine and *N,N*-dimethyl-1-decanamine were detected. In dichloromethane extracts additionally triethylcitrate and a couple of carboxylic acid esters were tentatively identified. With sitosterol, propylparaben, octyl-methoxycinnamate and probably some phthalates, EDCs were found in the influent.

In the effluent, some alkanes – although most of them not identified – were present. They might originate from skin care products like oils and creams which often contain mineral oils (hydrocarbons) instead of natural oils. The isoprenoid squalene is a naturally occurring alkene (e.g. in olive oil) which can also be an ingredient of skin care products. As it is also used as food additive, it may pass from kitchen sinks and automatic dish washers to the greywater stream to reasonable extents explaining it to be a "major" constituent of the influent. As it is also one of the predominant particular organics in the effluent, it is obviously not degraded very readily according to its occurrence in municipal secondary effluent (Li et al. 2005).

Within the group of alcohols/ethers, the substance diethylene glycol with both functional groups was found in the effluent. Besides spilling of deicing agents into sinks, the occurrence of this substance might be explained by the use of counterfeit tooth paste containing diethylene glycol as glycerol substitute. Three different polyethylene glycols, alcohols/ethers with longer chains than diethylene glycol, were also found to be present in the treated greywater. Polyethylene glycols are ingredients of skin cleaners as well as of oven cleaners.

Two phenols were detected in the effluent. *Tert.*-butylmethoxyphenol, also termed butylhydroxyanisol (BHA), is a common antioxidant contained in creams, ointments and lipsticks as well as in several industrially prepared foodstuffs. It represented a major compound in the effluent although not detected in the influent. With BHA an EDC was detected in biologically treated greywater. Di-*tert.*-butylphenol was found to be the main constituent in the solid phase extract of the effluent, but not in the dichloromethane extract. The main application of 2,6-di-*tert.*-butylphenol is its use as UV stabiliser and antioxidant for fuels, oils, gasolines and other hydrocarbon based products. Its occurrence in greywater might be referred to its appearance in hydrocarbons (mineral oils) used for fabrication of skin care products (see above). Its known recalcitrance may lead to its predominant occurrence among the trace organics of the constructed wetlands' effluent.

Another interesting result is that three ketones exhibited major peaks in the effluent SPE chromatograms. While acetophenon is clearly a fragrance ingredient, the origin of the other two is less clear. It remains speculative whether di-*tert.*-butyloxa-*spiro*-decadienedione is an oxidation product of a fragrance, as substances like 2,7-dimethyl-10-(1-methylethyl)-1-oxa-*spiro*[4,5]deca-3,6-diene are contained in fragrance ingredient databases. Di-*tert*-butylbenzoquinone is mentioned as a contaminant of the antioxidant TBHQ.

In accordance with Eriksson et al. (2003), long-chain carboxylic acids represent the predominant portion of gas-chromatographically detectable organics in untreated greywater (main peak in the influent chromatograms: palmitic acid). Contrasting to the terminology of Eriksson et al. (2003), palmitic acid cannot be looked at as a xenobiotic because it is a natural product derived from palm tree oil. That palmitic acid was also detected in the effluent indicates that the retention time in the planted soil filter is not sufficient for removing this highly concentrated biodegradable organic completely from the greywater.

Among the carboxylic acid esters, especially the phthalates (most of them not exactly identified) are of concern because some of them are known as EDCs. Moreover, the phthalate with a retention time of 20.35 min represented a major peak in the effluent solid phase extract and was the main peak in the liquid extract. One application of phthalates is fragrance enhancing. However, the "Phthalate Information Center" claims that the particular phthalate used as solvent in fragrances "is not linked to reproductive damage in humans". Another source of phthalates in greywater may be household dust ending up in greywater due to wiping up, because household dusts contain phthalates in reasonable concentrations (Riechelmann et al. 2007). A phosphoric acid ester found as a major contaminant in the effluent following SPE is tris-(1-chloropropyl)-phosphate, a flame retardant. It is contained in garment due to contamination by emissions from e.g. heat insulating materials and has been detected in the µg/l range in clothes washers' effluents (Proesch and Puchert 2003). Because of its persistence it is an organic of concern in the aquatic environment.

Finally, some nitrogenous compounds were found in the constructed wetlands effluent. The benzotriazoles probably originate from dishwashing agents, while benzoquinoline was found to be formed during grilling of chicken meat (Janoszka et al. 2004) and may be introduced into greywater via kitchen sinks and automatic dishwashers. The source of the acephylline derivative found as a major peak in the chromatogram of the effluent solid phase extract can be assumed to be a bronchodilator contained in cough syrups.

CONCLUSION

Regarding trace organics, biologically treated greywater resembles secondary municipal effluents. As also EDCs and a persistent flame retardant have been tentatively identified, discharge of biologically treated greywater to water bodies as well as to soils might be of similar concern as discharge of secondary effluents. With respect to trace organics, re-use of biologically treated grey water for purposes like car-washing or toilet-flushing does not seem to be a problem. Ecotoxicological evaluations will have to clarify whether advanced treatment is required for other kinds of re-use like gardening or groundwater recharge. Several detected organics implicate their origin from household chemicals or personal care products. More rigid regulations on these products might disable particular trace organics to contaminate greywater. But also airborne emissions can contribute to trace organic contamination of greywater as the examples of phthalates (adsorbed to household dusts and transferred to greywater by wiping up floors) or the persistent flame retardant tris-chloropropyl-phosphate (adsorbed to garment and introduced into greywater by laundry) show. Trace organics of this origin can only be eliminated from biologically treated greywater by advanced treatment.

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