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Occurrence of emerging pollutants in waste electrical and electronic equipment (WEEE) and sewage sludge from Norway

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List of abbreviations

A

2-ABTH – 2-amino-benzothiazole

5-ABTR – 5-amino-benzotriazole

ABS – Acrylonitrile, Butadiene Styrene plastic

APCI – Atmospheric pressure chemical ionization

AR% – Absolute recovery

AREP – Advanced Recycling, Recovery, and Reuse

B

BTH – Benzothiazole

BTHs – Benzothiazoles

BTR – 1H-benzotriazole

BTR-COOH – benzotriazole-5-carboxylic acid

BTRs – Benzotriazoles

C

2-Cl-BTH – 2-chlorobenzothiazole

5-Cl-BTR – 5-chlorobenzotriazole

CLEVER – Closed Loop Emotionally Valuable E-Waste Recovery

D

DL – Disposable Liner

E

ECHA – European Chemicals Agency

ECOSAR – Ecological Structure Activity Relationships

ED – Endocrine disruptor

EEE – Electronic and electric equipment

EIP – European Innovation Partnership

EPA – Environmental Protection Agency

EPs – Emerging Pollutants

ESI – Electrospray Ionization

ESI+ – Electrospray Ionization in positive mode

E-waste – Electronic waste

F

FAO – Food and Agriculture Organization

FTIR – Fourier-transform infrared spectroscopy

G

GC – Gas chromatography

GPC – Gel permeation chromatography

H

HPLC – High-Performance Liquid Chromatography

I

ISWA – International Solid Waste Association

ITU – International Telecommunication Union

L

LC – Liquid chromatography

LLE – Liquid-liquid extraction

LOD – Limit of detection

logP – Logarithm of octanol-water distribution coefficient

LOQ – Limit of quantification

LSE – Liquid-Solid Extraction

M

2-M-BTH – 2-morpholin-4-yl-benzothiazole

2-Me-BTH – 2-methylbenzothiazole

2-MeS-BTH – 2-methylthio-benzothiazole

4-Me-BTR – 4-methylbenzotriazole

5-Me-BTR – 5-methylbenzotriazole

m/z – mass/charge ratio

MAE – microwave-assisted extraction

ME% – Matrix effect

MobileRec – Collection, Disassembly and Recycling of Mobile Telecommunication Equipment)

MR% – Method recovery

MRM – Multiple Reaction Monitory

MS – Mass Spectrometry

MS/MS – Tandem Mass Spectrometry

MSPD – Matrix solid-phase dispersion

N

NORMAN – Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances

NP – Normal phase

NSES – National Strategy for Electronic Stewardship

NTNU – Norwegian University of Science and Technology

NTP – National Toxicology Program

O

1-OH-BTR – 1-hydroxybenzotriazole

2-OH-BTH – 2-hydroxy-benzothiazole

OECD – Organisation for Economic Co-operation and Development

P

PE – Polyethylene

PET – Polyethylene terephthalate

PFCs – Perfluorinated compounds

PFPE – Perfluoropolyether

PHWW - Pressurized hot-water extraction

PLE - pressurized liquid extraction

PNEC – The Predicted No Effect Concentration

PP – Polypropylene

PS - Polystyrene

PUR – Polyurethane

PVC - Polyvinyl chloride

Q

Q_1 – Transition of quantification

Q_2 – Transition of confirmation

QqQ – Triple quadrupole

QSAR – Quantitative structure-activity relationship

QTOF – Quadrupole-Time of Flight

QuEChERS - Quick, Easy, Cheap, Effective, Rugged and Safe

R

RT – Retention time

RAS – Return activated sludge

RMR% – Relative method recovery

RP – Reverse phase

S

2-S-BTH – 2-mercaptobenzothiazole

2-SCNMeS-BTH – 2-thiocyanomethyl-thiobenzothiazole

SDA – Small Domestic Appliances

SPE – Solid-Phase Extraction

SRMs – Secondary raw materials

StEP – Solving the E-waste Problem

STP – Sewage Treatment Plant

T

TA – Target analyte

TTR – tolyltriazole

U

UAE – Ultrasonic-assisted extraction

UHPLC – Ultra High-Performance Liquid Chromatography

UNCRD – United Nations Centre for Regional Development

UNU – United Nations University

W

WAS – Waste activated sludge

WEEE – Waste electrical and electronic equipment

WW – Wastewater

WWTP – Wastewater treatment plant

X

XTR – Xyliltriazole

&

ΔE - Energy gap

ΣBTHs - Summatory of benzothiazole concentrations

ΣBTRs - Summatory of benzotriazole concentrations

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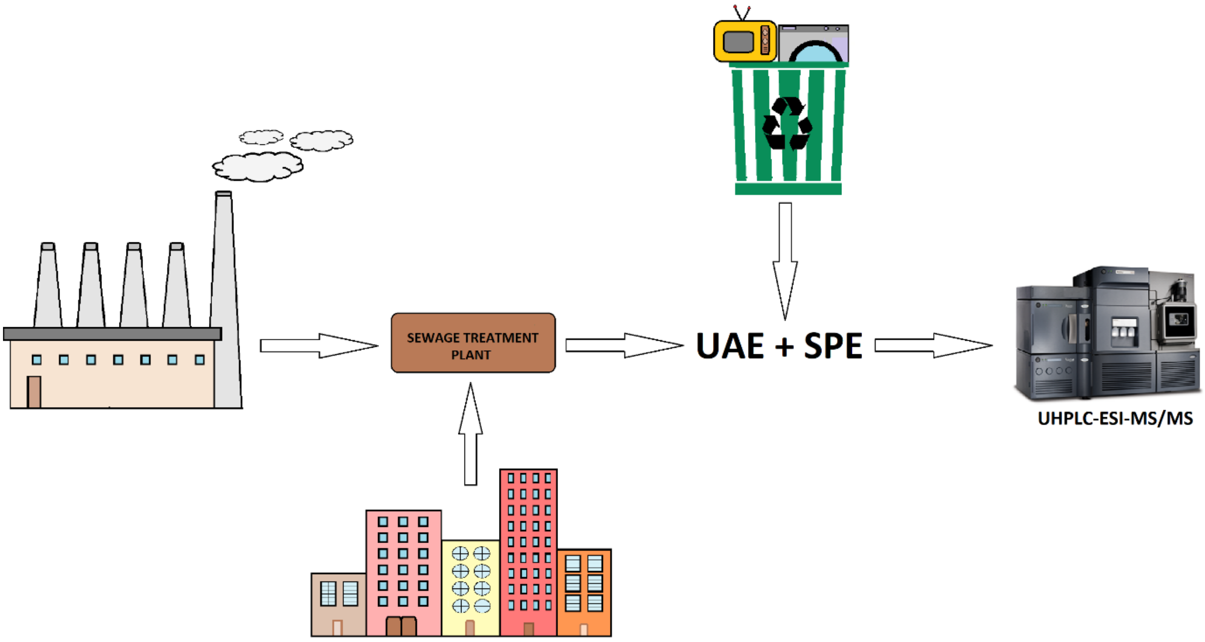
Graphical abstract



Abstract

Benzotriazoles (BTRs) and benzothiazoles (BTHs) are emerging pollutants ubiquitous in the environment due to their high production and wide use in industries and households. However, little information about their occurrence in the environment is available. In this project, the occurrence of 7 benzotriazoles, in particular, 1H-benzotriazole (BTR), xyliltriazole (XTR), 5-amino-benzotriazole (5-ABTR), 5-chlorobenzotriazole (5-Cl-BTR), 1-hydroxybenzotriazole (1-OH-BTR), benzotriazole-5-carboxylic acid (BTR-COOH) and tolyltriazole (TTR); and 9 benzothiazoles, i.e., benzothiazole (BTH), 2-amino-benzothiazole (2-ABTH), 2-mercaptobenzothiazole (2-S-BTH), 2-methylthio-benzothiazole (2-MeS-BTH), 2-hydroxy-benzothiazole (2-OH-BTH), 2-morpholin-4-yl-benzothiazole (2-M-BTH), 2-chlorobenzothiazole (2-Cl-BTH), 2-thiocyanomethylthio-benzothiazole (2-SCNMeS-BTH) and 2-methylbenzothiazole (2-Me-BTH) were studied in two different matrices, waste electrical and electronic equipment (WEEE) and sewage sludge, collected in different waste facilities in Norway. The proposed sample preparation included a liquid-solid extraction (LSE) assisted by ultrasound followed by a solid phase extraction (SPE) cleanup. Finally, the resulting extracts underwent an instrumental analysis based on ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UHPLC-ESI-QqQ). Out of the 16 initially proposed compounds, 11 were successfully validated in both matrices, which confirmed its

high versatility. The limits of detection ranged between 0.02 and 1.67 ng L^{-1}, and the linearity was satisfactory with R^2 in the ranging between 0.9905 and 0.9971 for the selected 11 compounds. Precision was satisfactory with a RSD% intra-day below 22%, in all cases.

The total concentration of BTRs in WEEE ranged between 3.8 and 496 ng g^{-1}, being BTR the pollutant displaying the highest concentrations, followed by TTR. Regarding BTHs, the concentrations measured in WEEE presented values between 102 and 9,695 ng g^{-1}, with the predominance of BTH. In sewage sludge samples, the occurrence and evolution of BTRs and BTHs during the treatment in the sewage treatment plants (STPs) showed a high persistence of TTR, XTR, BTR, 2-MeS-BTH and BTH. The evolution of some compounds as TTR or BTR differed between STPs, showing the differences between the treatments. Others like BTH and XTR were clearly degraded (or transformed). In contrast, 2-MeS-BTH showed to be very recalcitrant. The presence of high concentrations of BTRs and BTHs in the final sludge combined with its disposal in landfills or agricultural application as fertilizer, constitutes a serious environmental problem. Hence, using the data provided in this study, e.g., STP-3 would contribute with more than 2,300 and 5,000 kg y^{-1} of BTRs and BTHs, respectively, which could reach the environment affecting the wildlife and the crops.

More research about the occurrence and behaviour of BTRs and BTHs in sewage sludge is still needed in order to find out the extension of human and environment exposition to these compounds, and its consequences in the ecosystem health. This study provided more light about the issue, and presented a multi-residue methodology which concomitantly analyses a high number of BTRs and BTHs. To the best of our knowledge, the occurrence of BTRs and BTHs in WEEE was assessed for the first time in this work.

Resumen

Benzotriazoles (BTRs) y benzothiazoles (BTHs) son contaminantes emergentes presentes en el medio ambiente debido a su alta producción y su amplio uso en industrias y hogares. Sin embargo, aún existe poca información acerca de su presencia en el medio ambiente. En este trabajo, la presencia de 7 benzotriazoles, entre los que se encuentran 1H-benzotriazole (BTR), xyliltriazole (XTR), 5-amino-benzotriazole (5-ABTR), 5-chlorobenzotriazole (5-Cl-BTR), 1-hydroxybenzotriazole (1-OH-BTR), benzotriazole-5-carboxylic acid (BTR-COOH) and tolyltriazole (TTR); y 9 benzothiazoles, incluyendo benzothiazole (BTH), 2-amino-benzothiazole (2-ABTH), 2-mercaptobenzothiazole (2-S-BTH), 2-methylthio-benzothiazole (2-MeS-BTH), 2-hydroxy-benzothiazole (2-OH-BTH), 2-morpholin-4-yl-benzothiazole (2-M-BTH), 2-chlorobenzothiazole (2-Cl-BTH), 2-thiocyanomethylthio-benzothiazole (2-SCNMeS-BTH) and 2-methylbenzothiazole (2-Me-BTH) fueron estudiados en dos matrices diferentes, en particular, residuos de aparatos eléctricos y electrónicos (WEEE) y lodos de depuradoras de aguas residuales, muestreadas en distintas instalaciones de residuos en Noruega. La preparación de muestra propuesta incluyó una extracción líquido-sólido (LSE) asistida por ultrasonidos, seguida por una etapa de limpieza basada en una extracción en fase sólida (SPE). Finalmente, los extractos se sometieron a un análisis instrumental por cromatografía de líquidos de ultra-alta resolución acoplada a un espectrómetro de masas de triple cuadrupolo (UHPLC-ESI-QqQ). De los 16 compuestos inicialmente propuestos, 11 fueron validados con éxito en ambas matrices, lo que pone de manifiesto la alta versatilidad de la metodología. Los límites de detección se encontraron en el intervalo de 0.02 a 1.67 ng L^{-1} y la linealidad presentó valores de R^2 en el rango 0.9905 y 0.9971 para los 11 compuestos. La precisión observada fue satisfactoria, con RSD% intra-day inferiores a 22% en todos los casos.

Las concentraciones totales de BTRs en WEEE se encontraron entre 3.8 y 496 ng g^{-1} siendo BTR el contaminante que mostró mayores concentraciones, seguido de TTR. En cuanto a los BTHs, las concentraciones medidas en WEEE presentaron valores entre 102 y 9,695 ng g^{-1} con predominio de BTH. En los lodos residuales, la presencia y evolución de BTRs y BTHs durante el tratamiento en las plantas de tratamientos de residuos (STPs) mostraron la predominancia de TTR, XTR, BTR, 2-MeS-BTH y BTH. La presencia de compuestos como TTR o BTR varió en función de las diferencias técnicas de cada STP. Mientras que analitos como BTH y XTR fueron consistentemente degradados (o transformados) en todos los casos. Por contra, 2-MeS-BTH

demostró ser altamente recalcitrante. La presencia de altas concentraciones de BTRs y BTHs en el lodo resultante después de su tratamiento en las STPs, junto con su descarga en vertederos o uso como fertilizante agrícola, constituye un serio problema medioambiental. Con los datos presentados en este estudio, el lodo tratado en la STP-3 contribuiría con más de 2,300 y 5,000 Kg y^{-1} de BTRs y BTHs, respectivamente, lo que podría afectar a la fauna salvaje y a los cultivos.

Más investigación sobre la presencia y evolución de BTRs y BTHs en lodos residuales es necesaria para conocer la extensión de la exposición humana y ambiental a estos compuestos y sus consecuencias en la salud de los ecosistemas. Este estudio arroja luz al problema, presentando un método capaz de analizar simultáneamente un elevado número de BTRs y BTHs. Hasta donde hemos podidos saber, este trabajo constituye la primera vez que los BTRs y BTHs son analizados en WEEE.

1. Introduction.

1.1 Emerging contaminants.

According to the NORMAN network (Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances), emerging pollutants (EPs) can be defined as ''pollutants that are currently not included in routine monitoring programmes at the European level and which may be candidates for future regulation, depending on research on their (eco)toxicity, potential health effects and public perception and on monitoring data regarding their occurrence in the various environmental compartments'' [1][2].

In 2016, the NORMAN network updated a list with more than 950 non-regulated substances which have already been detected in the environment. They are classified in families i.e., flame retardants, pharmaceuticals, industrial and household additives, pesticides and their derivatives, personal care products, endocrine disrupting compounds, biocides, surfactants as well as drugs of abuse, steroids and hormones, gasoline additives, nanomaterials, and swimming pool disinfection by-products, among others [1][3].

The study of these compounds and their health and environmental effects is required to establish suitable regulations to control their worldwide spread.

1.2 Justification and objectives.

It has been widely proven that EPs constitute a risk to the environment and human health [3][4]. Therefore, the concentration levels in the environment must be limited to avoid this impact. In this project, samples of sludge from STPs and waste electrical and electronic equipment (WEEE) were analyzed. Regarding to the sewage sludge, it is paramount to monitor the presence and concentrations of EPs in STPs to assess their removal efficiency. In Europe, 50% of the produced sludge in 2019 was applied as fertilizer in agriculture, while 28% was incinerated and 22% landfilled. In the particular case of Norway, 82% of the produced sludge was used in agriculture and only 1% was incinerated [5][6]. Hence, residues from fertilizers produced from sewage sludge may contaminate aquifers and/or reach to human food, while sludge incineration contributes to the climate change, and dumping constitutes an environmental management issue. Moreover, WEEE entails increasing concern too, as its disposal may introduce hazardous substances into the environment, which in combination with its high production level, could produce adverse effects to humans, wildlife and the environment [7].

The main objective of this project was to study the occurrence of different EPs in WEEE, and sludge samples collected from different recycling facilities and STPs located in Norway. In addition, the effect of different thermal treatments applied in the facilities was evaluated for the removal of the pollutants.

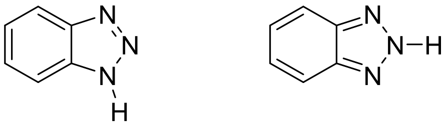
In this context, the application of one single sample preparation was assessed for the simultaneous extraction of several families of compounds, such as parabens, phthalates, perfluorinated compounds (PFCs), bisphenols, benzophenones, benzotriazoles (BTRs) and benzothiazoles (BTHs). In addition, it is worth pointing out that the same protocol was applied to both types of samples, sewage sludge and WEEE. Such method versatility would enable a faster and more cost-effective analysis. Finally, 6 BTRs and 5 BTHs were identified and quantified for both target matrices. The determination and quantification of the other families are not included in this manuscript. However, to the best of our knowledge, this is the first study focused on the determination of BTRs and BTHs in WEEE solid samples. Once again, as far as we known, this is the first study which monitored the occurrence of 2-M-BTH and 2-Me-BTH in sludge, and which reported distribution coefficients onto sludge for 1-OH-BTR, 5-Cl-BTR and 2-M-BTH.

1.3 Benzotriazoles and benzothiazoles.

Benzotriazoles **(BTRs)** and benzothiazoles **(BTHs)** are highly produced anthropogenic chemicals commonly used as corrosion inhibitors and complexing agents in a wide variety of products and industrial applications [8]. Both families have in common the presence of a nitrogen heterocyclic ring in their structure, and its structure and applications are explained below.

1.3.1 Benzotriazoles

The original compound of benzotriazoles, and the one that confers the name to the family, is 1-H-benzotriazole (BTR) which consists of a benzene fused to a 1,2,3 triazole ring containing 3 atoms of nitrogen. It exists in two tautomeric forms (Figure 1). At room temperature, 1a is the predominant form, where the hydrogen linked to nitrogen migrate easily between the non-consecutive nitrogen atoms conferring weak acid-base properties to the molecule [9].



1a 1b

Figure 1. Tautomeric forms of BTR.

Applications

The presence of **BTR and their derivates** in the environment is a consequence of their high stability and their wide use in household and industrial applications.

BTRs can establish a stable coordination in copper surface, thus they have been used as corrosion inhibitors since the end of 40s [9]. In addition, they are also currently used as flame inhibitors, in de-icing and anti-icing fluids, as ultraviolet light stabilizers in plastics, as silver protectors in dishwashing agents, as antifogging agents, as pigments and in dry cleaning equipment, among other applications [10] [11] [12]**.**

Studied BTRs

A total of 8 BTRs (Figure 2) were included in the present study. In particular; BTR, XTR (or 5,6 dymethyl-1H-benzotriazole), 5-ABTR, 5-Cl-BTR, 1-OH-BTR, BTR-COOH, 4-methylbenzotriazole (4-Me-BTR) and 5-methylbenzotriazole (5-Me-BTR). The mixture of 4-Me-BTR and 5-Me-BTR is commonly known as TTR [13].

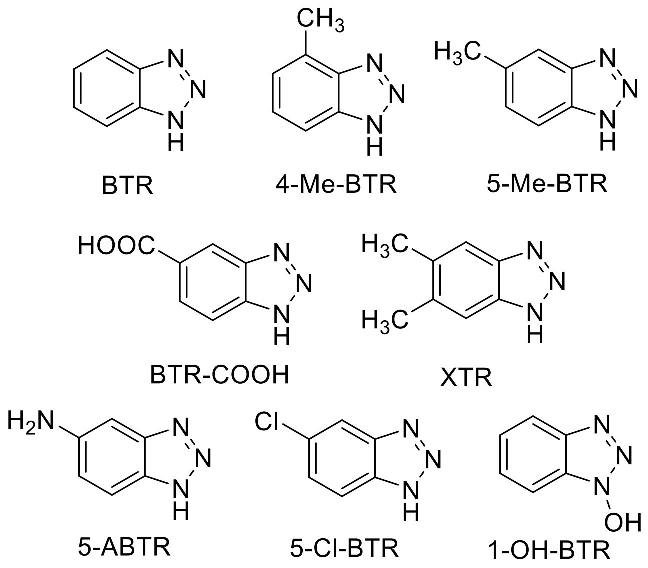
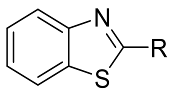


Figure 2. Benzotriazole (BTR) and its derivates.

1.3.2 Benzothiazoles.

Regarding to benzothiazoles, the simplest compound is formed by a benzene nucleus fused to a 1,3-thiazole ring, which consists of a five-ring structure containing a nitrogen and a sulfur atom. Their derivates are the result of adding diverse functional groups, which are generically expressed as 'R' in Figure 3.



1,3-Benzothiazoles

Figure 3. Generic molecular formula for BTH family where 'R' refers to different chemical groups.

Applications.

BTHs are widely used as rubber products to accelerate vulcanization and to enhance mechanical strength and abrasion resistance [14]. They are also used as corrosion inhibitors, herbicides, antialgal agents, slimicides in paper and pulp industry, photosensitizers, constituents of azo dyes, in de-icing/anti-icing fluids, antitumor reagents, fungicides in lumber and leather production [15], in pharmaceutical synthesis [16] among other applications.

Studied BTHs.

A total of 9 BTHs (Figure 4) were included in the present study. In particular; BTH, 2-ABTH, 2-S-BTH, 2-MeS-BTH, 2-OH-BTH, 2-M-BTH, 2-Cl-BTH, 2-SCNMeS-BTH and 2-Me-BTH.

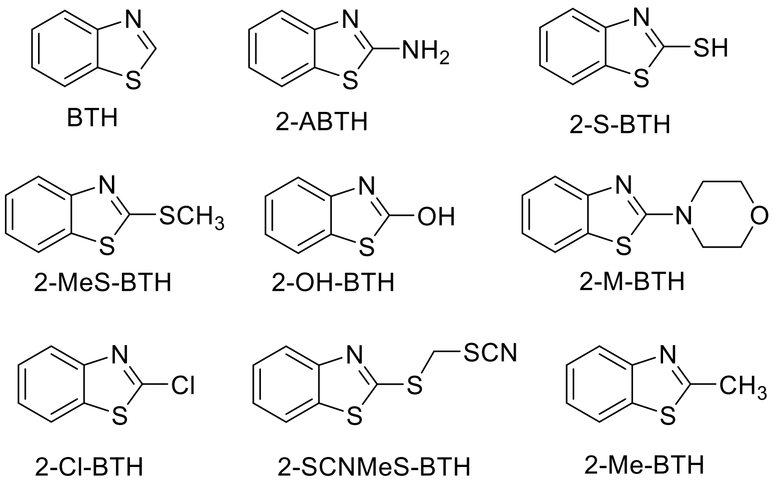


Figure 4. Benzothiazole (BTH) and its derivates.

Table 1 summarizes some of the BTRs and BTHs physico-chemical properties.

Table 1. Target analyte (TA) physico-chemical properties.

Name	Abbreviature	CAS	Formula	M.W. (Da)[a]	pKa [b]	LogP [c]
Benzothiazole	BTH	95-16-9	C_7H_5NS	135.186	0.85±0.10	2.169
2-Mercaptobenzothiazole	2-S-BTH	149-30-4	$C_7H_5NS_2$	167.251	9.80±0.20[d]	2.862
2-Hydroxybenzothiazole	2-OH-BTH	934-34-9	C_7H_5NOS	151.186	10.41±0.20 [d]	2.353
2-Aminobenzothiazole	2-ABTH	136-95-8	$C_7H_6N_2S$	150.201	3.94±0.10 [e]	2.002
2-(Methylthio)benzothiazole	2-MeS-BTH	615-22-5	$C_8H_7NS_2$	181.278	1.22±0.10 [e]	3.225
2-Morpholin-4-yl-benzothiazole	2-M-BTH	4225-26-7	$C_{11}H_{12}N_2OS$	220.291	3.19±0.10 [e]	2.71
2-chlorobenzothiazole	2-Cl-BTH	615-20-3	C7H4ClNS	169.631	-0.23±0.10 [e]	2.814
2-(Thiocyanomethylthio)benzothiazole	2-SCNMeS-BTH	21564-17-0	$C_9H_6N_2S_3$	238.352	-0.09±0.10 [e]	3.118
2-Methylbenzothiazole	2-Me-BTH	120-75-2	C_8H_7NS	149.213	1.65±0.10 [e]	2.716
1H-Benzotriazole	BTR	95-14-7	$C_6H_5N_3$	119.124	8.38±0.10[d]	1.167
4-Methyl-1H-benzotriazole	4-Me-BTR	29878-31-7	$C_7H_7N_3$	133.151	8.74±0.40 [d]	1.714
5,6-Dimethyl-1H-benzotriazole monohydrate	XTR	4184-79-6	$C_8H_9N_3$	147.177	8.92±0.40 [d]	2.261
Benzotriazole-5-carboxylic acid	BTR-COOH	23814-12-2	$C_7H_5N_3O_2$	163.133	3.47±0.30 [d]	1.048
5-Chlorobenzotriazole	5-Cl-BTR	94-97-3	$C_6H_4ClN_3$	153.569	7.46±0.40 [d]	1.811
1-hydroxybenzotriazole	1-OH-BTR	2592-95-2	$C_6H_5N_3O$	135.123	7.39±0.58 [d]	0.1095
5-Aminobenzotriazole	5-ABTR	3325 11 9	C6H6N4	134.139	9.61±0.40 [d]	0.2499
5-Methylbenzotriazole	5-Me-BTR	136-85-6	C7H7N3	133.151	8.74±0.40 [d]	1.714

[a]Source: Chemspider.com. [b]Source: Scifinder. [c]Predicted by ECOSAR 2.0 (EPA). [d]Strongest acid pKa. [e]Strongest basic pKa.

1.3.3 Background

BTRs are stable substances versus acids, alkalis, oxidation and reduction [17]. Consequently, they are expected to persist in water and, in general, in any environmental compartment [18]. Likewise, BTHs have been consistently identified in environmental waters in several studies as well [19][20]. Hence, recent studies reported median concentrations of BTRs and BTHs of 15.6 ng L^{-1} and 406 ng L^{-1}, respectively, in **tap water** from 51 cities in China [21]. In addition, TTR was found in concentrations up to µg L^{-1}, in **effluent and influent wastewaters and sewage sludge** [10][22][23][24]. Moreover, previous studies showed that treatments at the STPs are not efficiently removing these compounds from the wastewater, and as a result, they are continuously released into continental waters such as rivers and lakes, among others [10][23][25][26][27][28]. BTR and TTR [29][30][31] are the predominant BTRs in **rivers, lakes and natural sea waters**, as well as BTH [19]. **In ground waters**, BTR and TTR have been found in around 50% of the studied samples, in concentrations up to 1,032 and 516 ng L^{-1}, respectively [32].

Additionally, some analysis of BTRs and BTHs in **human samples** such as urine, amniotic fluid or adipose tissue have been published in the literature. Hence, the reported median concentrations (µg L^{-1}) in human urine (minimum/maximum) are summarized as follows: 1-OH-BTH (0.21/2.45); BTR (0.06/6.4); XTR (0.04/3.2); TTR (0.03/3.3); BTH (1.222/14.1); 2-OH-BTH (0.26/9.2); 2-MeS-BTH (0.24/0.33) and 2-ABTH (0.01/2.1) [11][16][33][34][35][36]. Additionally, BTH was found in amniotic fluid at median concentrations of 0.61 µg L^{-1}, and TTR, 5-Cl-BTH and 2-OH-BTH at 0.026 µg L^{-1}, 0.022 µg L^{-1} and 0.3 µg L^{-1}, respectively [36]. Some studies have informed about the presence of 2-OH-BTH as the main BTH in adipose tissue, followed by the BTRs: TTR and XTR [37].

Regarding biota, BTHs have been observed in **mollusks** with concentrations between 229-13,800 ng g^{-1} (dry weight), where BTH contributed with an 83.0%. Accumulated concentrations of BTRs ranged between 7.19 and 332 ng g^{-1}, with a 78.5% predominance of XTR, 5-Me-BTR and 1-H-BTR [38]. In addition, BTR has been found in average concentrations from 40 to 75 ng g^{-1} in different species of **fish** [39]

Finally, BTRs and BTHs have been monitored in **indoor and outdoor dust**, and 2-OH-BTH and XTR dominated in many cases [14][8][40]. Furthermore, a study of indoor dust in e-waste from a dismantling area in Qingyuan (China) showed accumulated concentrations of BTRs and BTHs of 3,830 ng g^{-1} and 2,070 ng g^{-1}, respectively, which are considerably higher than the concentrations found in other related dust studies [41].

1.3.4 Health and toxicology.

The National Program of the U.S. published in 1978 a bioassay of BTR for possible carcinogenicity, but the results did not show a clear evidence [42]. Later, the Committee of the Health Council of the Netherlands observed that BTR is an eye and a skin irritant, producing mutagenic effects in *Salmonella typhimurium* and in *Escherichia Coli*, and was categorized as injurious substance in case of inhalation or oral ingestion. It has been also classified as a suspected human carcinogen in previous studies [17][22].

Some other sources assured that BTHs could cause negative effects on the kidney and liver, and it is related to dermatitis and eye, skin, and respiratory irritation [43][44]. Furthermore, it is involved in microorganism mutagenicity, human cancerogenic [33] and aquatic toxicity [43].

It has also been observed that BTRs and BTHs are also toxic to luminescent bacteria, plants, and aquatic animals [45] and may have endocrine disruption properties [33]. Endocrine disruptors (EDs) are chemicals which alters the endocrine system and cause harmful effects in an organism or to its descent. There are indicators that BTR has endocrine disruptor properties which have arisen concern in the scientific community, but more research is still needed [46][47].

The predicted no effect concentration (PNEC) is defined as the concentration for which no adverse impact is expected in a particular species [48]. According to the NORMAN network, it is presented the lowest value among the PNECs predicted by QSAR for different species in freshwater (*Daphnid magna* and *Selenastrum capricornutum*) (Table 2) [1]. Additionally, the median lethal dose (LD50) for a substance is the dose required to kill half of the members in a tested population after a specified test duration. It is commonly predicted for fish and daphnia by the Ecological Structure Activity Relationships (ECOSAR). These parameters have been estimated for the target analytes (TA) in the present study and they are shown in Table 2.

Table 2. Toxicity parameters: Predicted LD50 (mg L^{-1}) in fish and Daphnid (ECOSAR model - EPA) and lowest PNEC (µg L^{-1}) for aquatic species.

	BTH	2-S-BTH	2-OH-BTH	2-ABTH	2-MeS-BTH
LC50 Fish (mg L^{-1})	78.3	1.57	3.79	16.2	11.8
LC50 Daphnid (mg L^{-1})	45.2	0.336	7.22	1.56	7.52
Lowest PNEC (µg L^{-1})	30	0.76	14	1	1
	2-M-BTH	2-Cl-BTH	2-SCNMeS-BTH	2-Me-BTH	BTR
LC50 Fish (mg L^{-1})	41.7	25.9	0.024	27.9	40.7
LC50 Daphnid (mg L^{-1})	25.3	15.9	0.028	16.9	244
Lowest PNEC (µg L^{-1})	14	1.14	0.38	1.92	7.77
	XTR	BTR-COOH	5-Cl-BTR	1-OH-BTR	5-ABTR
LC50 Fish	11.3	655	21.8	195	160
LC50 Daphnid (mg L^{-1})	37.6	4180	92.3	2060	1,530
Lowest PNEC (µg L^{-1})	4.94	16.9	3.57	- [a]	- [a]

[a] Not found.

1.3.5 Production

Currently, the global production of BTRs reaches 9,000 tonnes year^{-1} approximately [49]. The Environment and Climate Change Canada Health has recently published a ‘Draft Screening Assessment of the Benzotriazoles and Benzothiazoles Group’ including the total import of TTR and BTR in 2014 or 2015, reporting between 10,000 and 100,000 Kg of each compound per year [50].

The US National Toxicology Program (NTP) regularly summarizes the production of BTH from different sources. Hence, in 1993, United States produced between 4,500 and 45,000 Kg, and from August 1995 to October 1996, the import of BTH reached 203.9 Kg [51].

According to the European Chemicals Agency (ECHA), BTH is annually manufactured and imported to the European Economic Area between 10 to 100 tonnes [52].

1.4 Environmental samples.

In the present study, the occurrence of BTRs and BTHs was investigated in sewage sludge and WEEE samples collected in different areas of Norway. Information about the composition, regulation, production, and origin of these matrices is shown below.

WEEE

Definition

According to the European Union Law [53] electric or electronic equipment (EEE) can be defined as ‘‘equipment which is dependent on electric currents or electromagnetic fields in order to work properly, and equipment for the generation, transfer and measurement of such currents and fields which is designed for use with a voltage rating not exceeding 1,000 volts for alternating current and 1,500 volts for direct current’’. The same system of rules defines the waste electrical and electronic equipment (WEEE) as ‘‘electrical or electronic equipment which is waste within the meaning of Article 3(1) of Directive 2008/98/EC, including all components, subassemblies and consumables which are part of the product at the time of discarding’’. And within the Article 3(1) of Directive 2008/98/EC, waste is defined as ‘‘any substance or object which the holder discards or intends or is required to discard.’’

Similarly, electronic waste (e-waste) can be defined as any discarded device containing electronic circuits, such as transistors, capacitors, resistors, etc. They include domestic appliances (refrigerators, stoves, etc) and other electric apparatus, even if they are mainly made of plastics or steel [7]. However, in the recycling industries, the different components of e-waste are usually separated into fractions such as plastics, cables, metals, batteries, …

Even if WEEE and e-waste present slight differences (i.e., e-waste always contains electronic circuits and WEEE includes all components), it is usually used indistinctly in the literature.

Composition, classification and disposal

It has been estimated that around 8% of the total waste corresponds to WEEE [54]. The components of WEEE may contain dangerous substances for the environment and health, but also precious materials. Steel and iron, non-flame retarded plastics and other metals constitutes the most important groups of materials and the separation of the components is highly expensive [7]. As a consequence, recycling is complicated but necessary.

A classification of WEEE has been accomplished by the European Union, resulting into the following categories[55]:

- Huge domestic electric appliances
- Small domestic electric appliances
- Information technology equipment

- Media/entertainment/recording equipment
- Lightning appliances
- Electric construction tools of wood and metal
- Toys/leisure equipment along with sport items
- Medical equipment
- Surveillance equipment
- Electric dispensers

Landfills and incinerators are the principal end-point locations for discarded WEEE. Leachates in dumps are not always properly treated and may finish in the environment, with its subsequent contamination. Incinerators usually produce electricity, but also toxic and green-house-effect gases, which contributes to air pollution and climate change [7].

Production.

The production of WEEE during the last decades has dramatically increased, which has arisen major concern in the scientific community. The so-called ‘‘WEEE generated’’, is an indicator of the amount of WEEE consumed in a specific time and region, prior to any collection, reuse, treatment, or export. Figure 5 shows the WEEE generated per continent between 2015-2019.

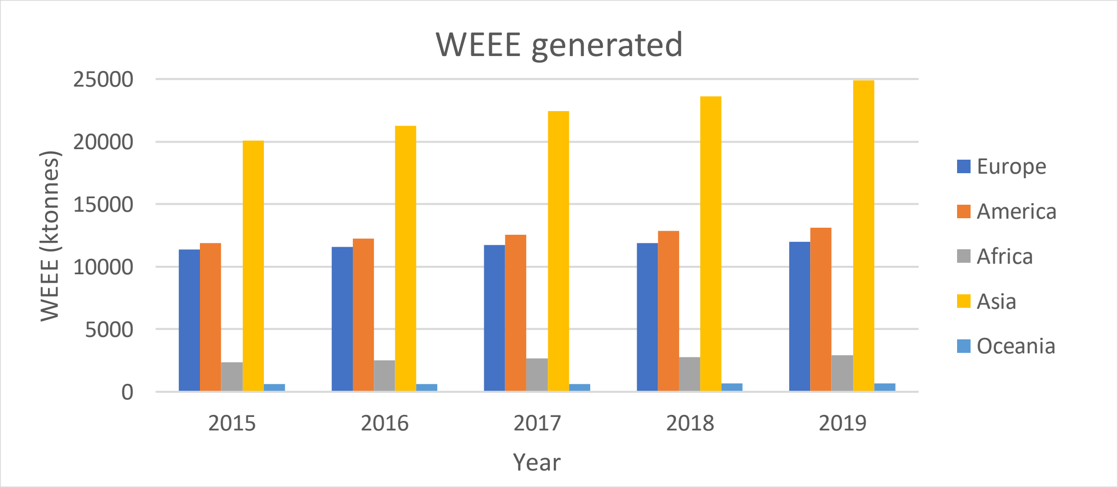


Figure 5. WEEE generated per year and continent between 2015-2019. Data from: Baldé C.P., Forti V., Gray, V., Kuehr, R., Stegmann,P. : The Global E-waste Monitor – 2017, United Nations University (UNU), International Telecommunication Union (ITU) & International Solid Waste Association (ISWA), Bonn/Geneva/Vienna. [56]

Additionally, the ‘‘WEEE formally collected’’ (WEEE collected and managed under specific WEEE regulations) is an indicator of how much WEEE is being recycled. Hence, some data of ‘‘WEEE collection rate’’ (WEEE formally collected/WEEE generated) in percentage has been reported for each continent: 43% (Europe, 2017), 9% (Oceania, 2018), 12% (Asia, 2016), 1% (Africa, 2016), 9% (America, 2016) [56].

Regulation.

Due to this growing problem, legislation and initiatives have been established. In 1989, the Basel Convention banned the free movement of hazardous waste. The countries belonging to the Organisation for Economic Co-operation and Development (OECD), the European Union (EU) and Japan assumed numerous regulations about WEEE in the first years of the 21st century. The WEEE Directive (2012/19/EU) regulates most of the WEEE in the European Union and Norway. Around a 42.5% of WEEE has been collected and recycled in Europe in 2019 [57]. Other examples of legislation and initiatives are from OECD with the 'Environmentally Sound Management of water: Reclaim E-Waste' in 2003 or the National Strategy for Electronic Stewardship (NSES) with the 'Improve design of electronic products' in 2011. Moreover, the United Nations (UN) promoted the initiative 'Solving the E-Waste Problem' (StEP,) and the UK presented the 'EEE sustainability action plan 2014' and 'CLEVER' [54]. In general, all these regulations aim to achieve a suitable environment and avoid the overproduction of electronic waste. As an example, one of the mentioned initiatives, StEP, promotes to reduce environmental impact associate to the e-waste management, by decreasing its production and promoting less hazardous and repairable new designs in order to extend their useful life [58][59].

Sewage sludge

Definition

Sewage sludge is recognized as a sink of many moderate and highly lipophilic persistent contaminants. For that reason, its production and impact in the environment have been a matter of concern for the scientific community during the last decades. It is a complex material considered as a solid/liquid residue. The density of this material can be described from fluid liquids principally made of water, to liquid-solid mixtures which have a wet-soil texture and are mainly solids [60].

The main source of residual sludge is the sewage treatment plants (STPs), where urban and industrial wastewater is treated. In addition, residual sludge can be produced in septic tanks during the management of sewage [61], as well as the sludge produced in aquaculture during the deposition of the non-eaten feed and fish excretion [62].

STPs

Sewage treatment plants (STPs), also known as 'wastewater treatment plants' (WWTPs) are facilities where wastewater is exposed to mechanical, physical, chemical and/or biological treatment to produce suitable products to discharge into the environment [63]. Sewage from health centres, industries and households from towns and cities must be treated before being discharged into the environment.

The Environmental Protection Agency (EPA) in the report EPA-540/R., Volume 93, Number 30 [64] provided the following definitions:

- Influent: ''water, wastewater, or other liquid flowing into a reservoir, basin, or treatment plant.''
- Effluent: ''wastewater, treated or untreated, that flows out of a treatment plant, sewer, or industrial outfall. Generally, refers to wastes discharged into surface waters.''

The design of each STPs depends on the contaminants presents in the influent, the applying regulations, and the destination of the effluent and the other byproducts. In general, they include an influent pre-treatment step, followed by a primary and secondary treatment and a final disinfection. A generic scheme is shown in Figure 6 .These steps are described in more detail as follows [65][66]:

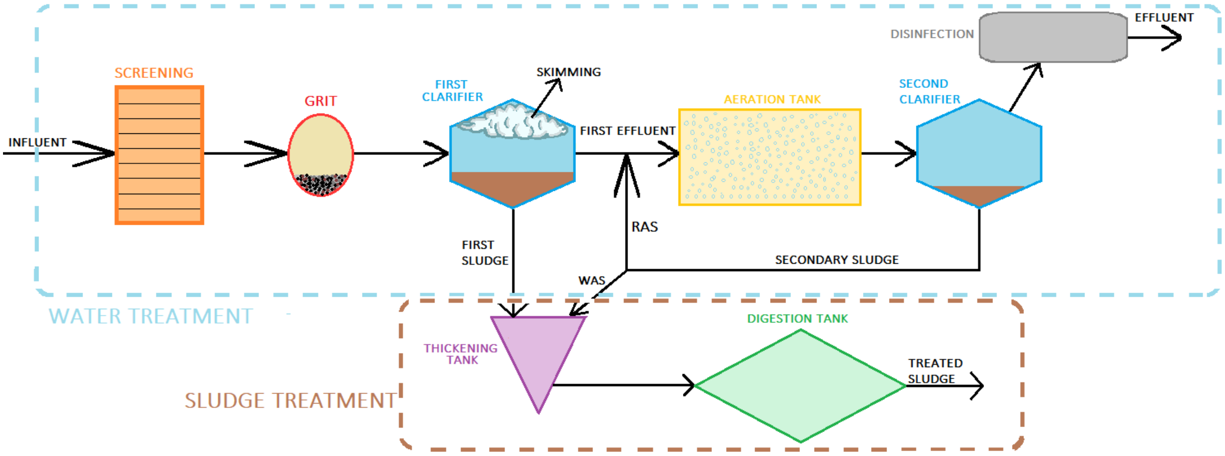


Figure 6. Generic design of a STP.

Pre-treatment. The influent could contain a variety of large solids such as wood, cans, rocks, plastics, etc. These materials are removed during this initial screening. Usually, a grit removal is applied. Colloidal and dissolved particulates are not separated yet.

Primary treatment. Organic and inorganic suspended solids are removed by sedimentation during this step, and the aftermath slurry, which is called ‘‘primary sludge’’, goes to the thickening tank. At the same time, floating substances, such as oils, microplastics, soap, etc. form a scum, which is eliminated by skimming. This step takes place in the first clarifier and the resulting water is transported to the aeration tank to undergo the secondary treatment described below. According to EPA, an average of 2,500 to 3,500 L of primary sludge is obtained per million of urban wastewater (WW) in conventional STPs. Furthermore, this primary sludge is made by a 3 to 7% solid, and 60 to 80% of it is made of organic matter [67].

Secondary treatment. The primary influent arrives to the aeration tank where an aerobic biological treatment typically takes place. A high concentration of dissolved oxygen is needed for the water-treating microorganisms to work. Thus, an air flow is continuously diffused into the water, stirring the water too. Organic and inorganic dissolved matter is consumed by and/or adsorbed onto the biomass purifying the water. Then, the resulting water reaches the sediment tank (secondary clarifier) where a sedimentation process takes place. The supernatant liquid, i.e., the effluent, is separated from the settled biomass. This latter constitutes the ‘‘secondary sludge’’ and is partially recirculated to the aeration tank to replenish the microbiotic level in the secondary treatment. The rest is conducted to the thickening tank. This process is known as ‘‘activated sludge’’ and is the biological treatment most commonly applied in municipal urban STPs around the world. EPA estimates that this technology is responsible for up to 90% of organic matter removal, producing secondary sludge with a typical content of solids ranging from 0.2 to 0.5% in volume (with a 50-60% of organic matter). During this step, between 15,000 to 20,000 L of secondary sludge are produced for each million L of effluent WW.

Tertiary treatment. Some STPs implement additional water treatments before it is released into the environment or reclaimed for agricultural applications, among others. This step includes advanced oxidation processes, such as disinfection with UV light or chlorine [68][69].

Sludge treatment. Primary and secondary sludge are usually mixed and thickened by centrifugation, diminishing the water content. This decreases the managing costs (transportation, volume to treat, etc). Then, the resulting sludge undergoes anaerobic biological digestion which reduces the sludge volume, toxicity and odour, and the biogas produced is used to cover the energy balance during the whole wastewater treatment. The resulting product is commonly used as fertilizer in the agriculture industry. Nonetheless, many local regulations limit this practice pointing out to the presence of remaining harmful agents, both chemical and biological. Thus, sludge is often disposed into landfills too. Most harmful microorganisms are destroyed a pH above 11.5. The addition of calcium hydroxide to control the pH is a common practice when the sludge is intended to be reused as fertilizing biosolid [70]. Further research is required to develop suitable technologies to turn sewage sludge into a safe valuable material, in accordance to the principles of the so-called circular economy.

Production, composition, and disposal

The annual production of sewage sludge for a selection of countries in 2018 according to the database ''Eurostat'' is shown in Figure 7.

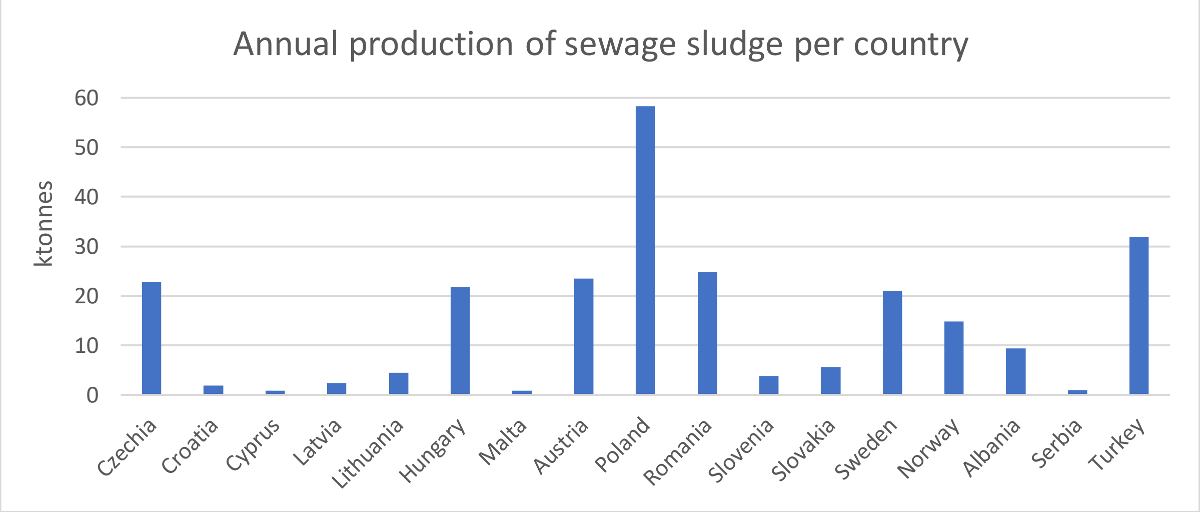


Figure 7. Production of sewage sludge in ktonnes in 2018 in several countries. [70]

Organic matter, N, P and K are the principal components of sludge, but also heavy metals as Zn, Cu, Pb, Hg, Ni, Mo, Cr, or even pharmaceuticals and personal care products occur in concentrations above ng g^{-1} [71][69]. The presence of heavy metals in sludge might entail a safety issue and hamper its use in agriculture. According to Food and Agriculture Organization (FAO) Cd, Pb, Hg and Ni are potentially injurious to humans, and the use of sludge in agriculture increases the levels of these metals in animals and plants [72]. Therefore, regulations limiting their concentrations have been set. Hence, the European Commission has established thresholds for Cd (20-40 µg g^{-1}), Cu (1,000-1,750 µg g^{-1}), Ni (300-1,200 µg g^{-1}), Pb (750-1,200 µg g^{-1}) or Zn (2,500-4,000 µg g^{-1}), all referred to as dry matter [61].

Furthermore, EPs are also present in the influent, effluent and sludge from STPs [73][74]. As they are non-regulated compounds, STPs are not specifically designed to obtain an efficient removal, and they are often uncontrolledly discharged into the environment. The presence of BTRs and BTHs, have been particularly reported in influents, effluents and sludge in several studies [28][23][27][75].

Final sludge management is one of the biggest challenges for STPs. Landfills are an option to return water, nutrients and organic matter from wet sludge to the environment, but also contaminants. Other options include thermal processing (incineration, combustion, pyrolysis, etc.) which affects the air quality [76], aggregation with other products, compost and forestry, among others [77]. Sludge has been even discharged in oceans and lagoons [78]. However, none of these practices are environmentally friendly. On the other hand, the current trend is to obtain economic advantages from this product instead of the simple ejection. In Norway, agricultural application as biosolid is the first destination of sewage sludge (Figure 8).

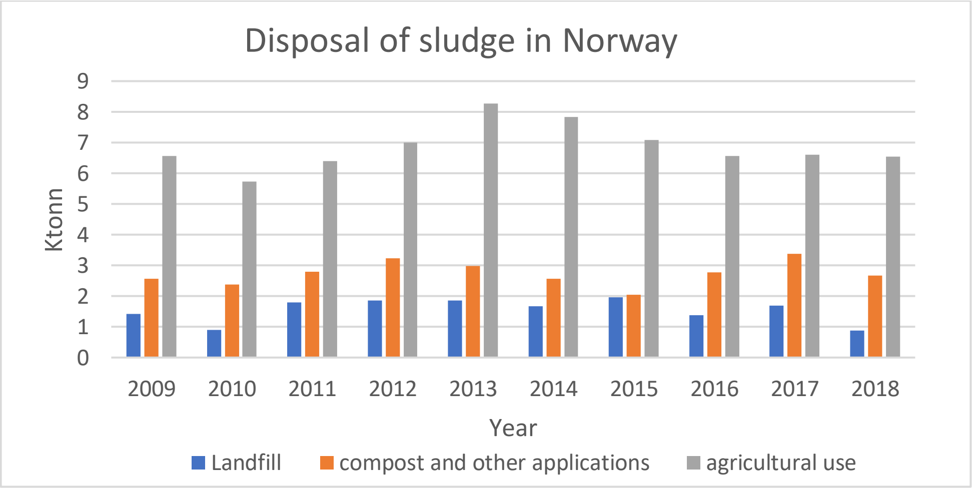


Figure 8. Destination of sludge in Norway in the last years. [70]

Environmental distribution

EPs are widespread across the world and affect the environment. The production of EEE products in industries are the first step to generate WEEE. Moreover, households and industries increase the amount of these kind of residues in the environment. Figure 9 shows the environmental distribution of EPs (applied to WEEE and sludge from STPs).

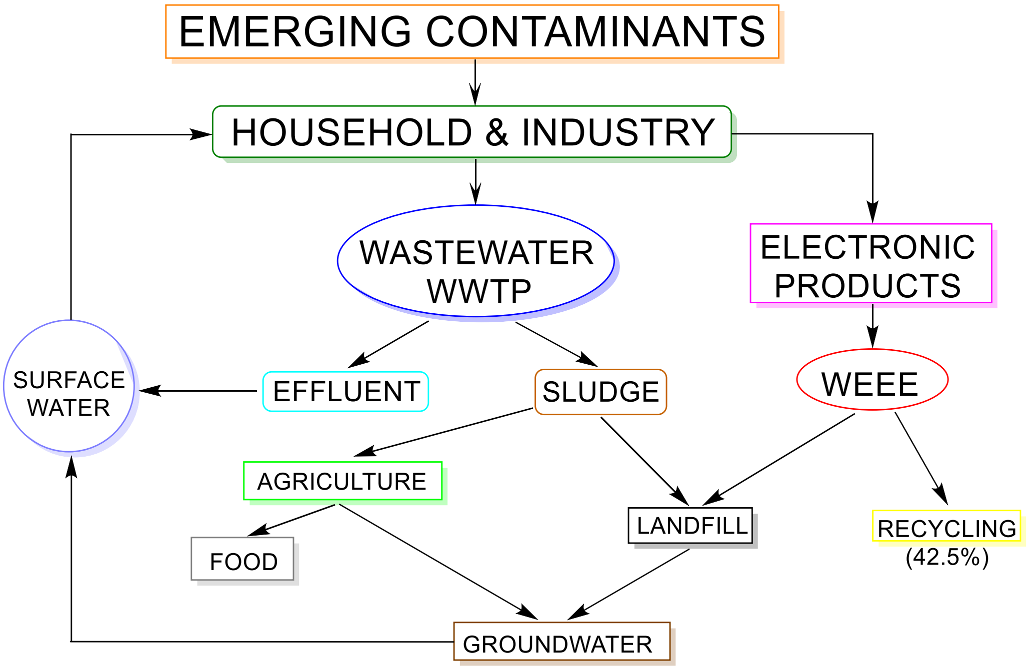


Figure 9. Environmental distribution of emerging contaminants. Applied to WEEE and sludge from STPs.

1.5 Theoretical background and method selection.

Many analytical methodologies for the analysis of EPs in different environmental matrices have already been developed by several research labs around the world. In all of them, matrix effect has entailed a challenge to face, as interferes in the analysis performance, especially in complex matrices such as sewage sludge and WEEE. Thus, a pre-treatment step is commonly carried out prior to the instrumental analysis.

The most common techniques for related sample preparation and instrumental analysis in sewage sludge are reviewed below. However, due to its novelty, little information is available in the scientific literature in the case of WEEE. Additionally, the main advantages and disadvantages are presented.

Sample preparation

Sample preparation is the most important step during the development of the analytical methodology as it is responsible for removing undesirable compounds from the matrix which may interfere with the target analytes during the analysis. The first pretreatment step may consist of a water removal. Centrifugation, decantation, air-drying and heating are options frequently used, but sample lyophilization is probably the most common to avoid uncontrolled evaporations or degradations [79]. Then, a liquid-solid extraction (LSE) usually takes place. LSE is not analyte-selective, and thus, a subsequent clean-up step is usually required [80].

LSE makes use of an appropriate solvent to extract the target compounds from a solid matrix. Temperate as well as type and volume solvent(s) have been reported to play a role in the analyte extraction selectivity [80].

Some of the most common extraction techniques used in the analysis of EPs in sewage sludge are the followings: soxhlet (or the automated soxhlet), UAE (ultrasonic-assisted extraction), MAE (microwave-assisted extraction), PLE (pressurized liquid extraction), MSPD (matrix solid-phase dispersion), PHWE (pressurized hot-water extraction) or QuEChERS (quick, easy, cheap, effective, rugged and safe) [81]. In the case of BTRs and BTHs, PLE, UAE, QuEChERS, PHWE or other conventional methods have been reported to be the most suitable for sewage sludge [82].

Solid phase extraction (SPE), gel permeation chromatography (GPC) or liquid-liquid extraction (LLE) are clean-up techniques which have been used after the extraction step described above. They are usually efficient in the removal of undesirable substances such as lipids, surfactants, etc. present in the sewage sludge matrix. SPE is a chromatographic extractive technique and has been reported to be the most commonly used to achieve this purpose in the sample extract [81]. Usually, it is performed in a cartridge with a solid phase (sorbent) whose properties and quantity determine the efficiency of the extraction. Due to the polarity of BTHs and BTRs, polymeric balanced polar/non-polar sorbents are used [82]. An initial cartridge activation and conditioning is required to eliminate impurities and optimize conditions in the cartridge (Figure 10). Usually, it is carried out by passing the same solvent that constitutes the sample. Then, the sample is passed through the sorbent which ideally retains the target analytes but not the interferences. After the selective retention, a cartridge rinsing and drying is usually conducted. Then, the elution of compounds is performed with an appropriate volume of a selected solvent. This clean-up protocol contributes to an extract concentration too, which is beneficial when working with low concentrated analytes, as it might be the case [80][83].

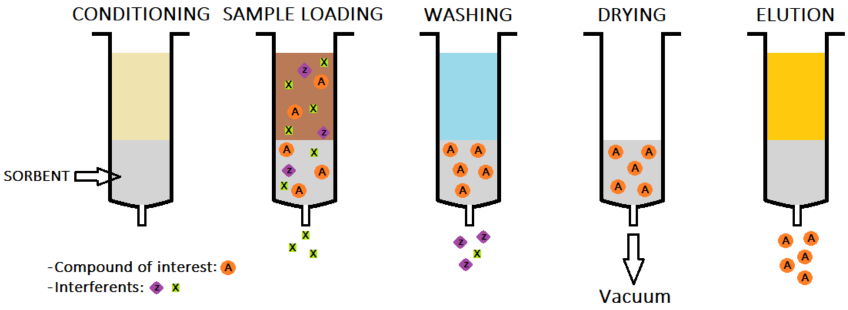


Figure 10. Common steps in SPE.

Instrumental analysis

Liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS) has been the predominant instrumental analysis techniques in the last years for analysis of EPs. GC requires analyte volatilization, which is not easy to achieve in the usually polar EPs without thermal degradation taking place. Derivatization may solve this limitation in some cases, but it entails additional sample manipulation. Thus, LC has been preferred over GC because of its versatility. [81][82]

In LC, the analytes are separated because of their different affinity between the mobile and the stationary phases, this latter usually supported in a column. Partition is the most common mechanism used in this analysis, and the analytes achieve separation by differential solution in the stationary and mobile phase, depending on their polarity and the chromatographic phase polarities. Analytes should hold polarities amidst the mobile and the stationary phase polarities, which will be far apart. Partition LC may be applied in two different modes, reverse phase (RP) and normal phase (NP), depending on the mobile phase polarity (and therefore stationary phase one too). Despite NP should be preferred for polar compounds, RP stationary phases usually deliver more precise chromatograms and therefore, RP is usually the preferred mode when possible. High-performance liquid chromatography (HPLC) enables an efficient separation by using packed columns with particle sizes of around 5 µm and high-pressure pumps. Additionally, smaller stationary-phase-particle sizes increase the peak resolution and the analyte sensitivity in the chromatograms. Nevertheless, it comes with an increasing instrument pressure. The development of UHPLC systems overcame this limitation, enabling the use of stationary phase particles <2 µm, which allowed the reduction of the time of the analysis as well as the requirement of solvent volumes [84].

Both LC and GC are separation techniques and need to be coupled to a detection instrumental technique to transduce a physical property related with the analyte concentration into an electric signal. Spectroscopic techniques such as UV-Vis or fluorescence were proposed as valid options at first. However, both sensitivity and selectivity are improved when mass spectrometry (MS) is used instead. In fact, mass spectrometry is the main detection technique in environmental samples, where the analytes are present at low concentration in very complex matrices [82]. In particular, in target analysis, tandem mass spectrometry (MS/MS) with a triple quadrupole analyzer is normally used in multiple reaction monitoring (MRM) mode. This consists of selecting a parent compound per analyte in the first quadrupole, which is fragmented in the collision cell and then, select a daughter ion to quantify. Both quadrupoles allows the transmission of a very narrow window of masses around the parent and daughter ion's, so it is very specific and sensitive [85]. In environmental samples, at least an extra qualification transition is usually needed to confirm. However, one of the main drawbacks of this approach is that it is completely blind to any other substances present in the sample. Other proposals include the detection of other molecules apart from the target analytes as they perform full mass scan by using high-resolution mass spectrometry with a quadrupole-time of flight spectrometer (QTOF) or an Orbitrap, for instance. In contrast, in these conditions, sensitivity and dynamic range decrease. Regardless, LC-MS/MS is heavily affected by matrix effect, both ion suppression and enhancement, and entails a challenge, especially when analysing traces in complex environmental samples such as sewage, sludge, etc. [82][81]. To produce ionization, first and for most electrospray ionization (ESI) but also atmospheric pressure chemical ionization (APCI) are typically used in both positive and negative ion mode [81]. ESI consist of applying a high voltage at atmospheric pressure onto an aerosol made of the mobile phase dissolving the analytes after chromatographic.

1.6 Working plan

The experimental analytical work was accomplished in the Norwegian University of Science and Technology (NTNU) under an Erasmus+ scholarship program.

Firstly, an evaluation of the sample pretreatment was assessed. Once the optimal conditions were selected, the whole method was validated. Finally, the developed methodology, based on UAE-LSE and SPE followed by UHPLC-ESI-MS/MS, was applied to real samples of WEEE and sewage sludge. Subsequent data processing of the resulting chromatograms was carried out and statistical treatment followed.

During the whole period, databases such as SciFinder and Scopes were checked for scientific literature. Finally, besides this present manuscript, at least one related scientific paper is expected to be published too.

2. Material and methods.

2.1 Chemicals and materials.

Analytical standards (purity) of 2-MeS-BTH (97%), 2-S-BTH (97%), 2-OH-BTH (98%), XTR (≥99%), BTH (≥97.0%), BTR-COOH (99%), 2-Me-BTH (99%), 2-ABTH (97%), 2-Cl-BTH (99%), 1-OH-BTR hydrate (≥97.0%), 5-Cl-BTR (99%), 5-Me-BTR (98%), 2-M-BTH (Aldrich^{CPR} grade), 5-ABTR (Aldrich^{CPR} grade) were purchased from Sigma Aldrich (Steinheim, Germany). 2-SCNMeS-BTH (98%) was purchased from Advanced ChemBlocks (Burlingame, USA). Labelled compounds used as internal standards (IS), 1H-Benzotriazole-(ring-d_4) solution (10 µg mL^{-1} in acetone) (BTR-d_4) was obtained from Sigma Aldrich and 5-methyl-1H-1,2,3-benzotriazole-d_6 (4,6,7-d_3 methyl-d_3) solution (100 µg mL^{-1} in methanol) (5-Me-BTR-d_6) was purchased from Chiron (Trondheim, Norway).

A standard solution mixture (1 mg L^{-1}) of all target analytes was prepared in methanol and stored at -20 °C for a maximum of a month. The same concentration was prepared for a solution of IS.

The following **solvents** (purity) were purchased in VWR chemicals (BDH PROLABO®, Fontenay-sous-Bois, France): methanol (≥99.8%) (MeOH) and acetonitrile (ACN) (≥99.9%) of LC-MS grade. Formic acid (FA) (≥96%) and hydrochloric acid (HCl) were acquired from Sigma Aldrich (Steinheim, Germany). Deionised water (Milli-Q grade water) was in-house obtained by a purification system (MILLIPORE S.A., Molsheim France).

Glass microfiber filters GF/F diameter 47 mm (Whatman^{TM}, Middlesex, U.K.) with 0.45 µm of pore size. They were used to filter some sludge samples and to accumulate particulate matter from these samples. An ALPHA 1-4 LD plus freeze dryer (Martin Christ; Osterode, Germany) was employed for dewatering solid sludge and particulate matter samples.

An automated solvent evaporation system (TurboVap® LV) from Biotage (Charlotte, U.S.A.), a Centrifuge 5810 (Eppendorf, Hamburg, Germany), a 3510 Ultrasonic Cleaner from Branson (Danbury, USA), a Vortex shaker (VWR, Bruchsal, Germany) and VWR® centrifuge tubes of 15 and 50 mL volume, relative centrifuge force (RCF) 12,500 g were used during the sample pretreatment.

For the clean-up step, a 24-port model Visiprep-DL Solid Phase Extraction vacuum manifold was purchased from Supelco (Bellefonte, PA, USA), as well as disposable flow control valve liners for the Visiprep^{TM}. SPE cartridges, Strata^{TM}-X 33 µm Polymeric Reversed Phase 200 mg / 6 mL were obtained from Phenomenex (Aschaffenburg, Germany).

2.2 Sampling and sample information.

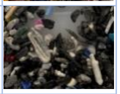
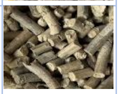
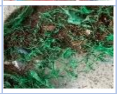
WEEE

A total of 15 samples of WEEE and plastic materials were collected in Norway from different facilities. They were coded as R1 to R9 and Q1 to Q6. Each sample and the process of collection is described below and summarized in Table 3.

R1-R9 samples were secondary raw materials (SRMs) arising from electronic waste collected in Norway and sieved after collection (<2 mm). SRMs consist of recycled materials that can be injected again into the economy [86]. The sampling date was December 2020. These SRMs are separated into different fractions which are described in Table 3. R1, R2 and R3 consist of small domestic appliances (SDA) which were separated according to the main kind of plastic they are made of i.e., acrylonitrile butadiene styrene (ABS), polypropylene (PP) polyethylene (PE) or polystyrene (PS). R4, R5 and R6 were the plastic components of different temperature exchange equipments (e.g., fridges). R7 was the reject fraction of SDA. Likewise, R8 is the reject fraction of R4, R5 and R6. On the other hand, R9 was an agglomerate of polyurethane (PUR) principally.

Q1 consisted of a mixture of PVC (polyvinyl chloride) and other plastics, collected from a Norwegian WEEE recycler in 2017. Q3 was polyethylene terephthalate (PET) plastic from drinking water bottles, and Q4 is ground PE and PP from bottle caps, both collected in October 2020. An innovating process was tested for biomass valorisation and calorific fraction of municipal solid waste. A mixture of Q3 and Q4, in proportions 9:1, was introduced in a hopper and then pyrolyzed (800 °C). The generated gas was, then, condensed as an oil and water. The resulting solid phase from the pyrolysis was cooled and collected as a sample (Q2). The plastics Q5 and Q6 were manually removed from the digested food waste in December 2020 and then, sieved. This could potentially be used as biofertilizer, but the presence of high amount of plastic entails a limitation. Q5 was mostly PE from green food waste bags collected in Oslo. Q6 was mainly made of biodegradable plastic from food waste bags (more than 50%) and the rest of the packaging (mix of PP, PS, etc.).

Table 3. WEEE samples. Code, category, mainly composition and image.

Sample code	Waste category	Main plastic type	Image
R1	Small equipment	ABS	
R2	Small equipment	PP/PE	
R3	Small equipment	PS	
R4	Temperature exchange equipment	ABS	
R5	Temperature exchange equipment	PE/PP	
R6	Temperature exchange equipment	PS	
R7	Small equipment	Mixed grinds	
R8	Temperature exchange equipment	Mixed grinds	
R9	Temperature exchange equipment/large equipment	PUR	
Q1	Norwegian WEEE recycler	PVC and others	
Q2	Char from pyrolysis Q3/Q4 (9:1)	PET/PE/PP	
Q3	Bottles	PET	
Q4	Bottle caps	PE/PP	
Q5	Plastic from food waste (bags)	PE	
Q6	Plastic from food waste (bags and packaging)	PE and others	

Samples Q2 to Q6 were included in the study due to a thermal analysis is being tested to see the removal efficiency of this EPs and to assess the thermochemical conversion of plastic.

Once the thermal analysis is probed with plastics from bottles, cap bottles and food waste plastics, it will be extended to WEEE samples due to the similarity of the materials. Additionally, this project included other families of EPs whose occurrence in these plastic samples (Q2 to Q6) was particularly interesting.

Sludge

Twenty-five grab samples from 3 different STPs in Norway have been collected in glass containers after different steps of the treatment. Then, when possible, samples were filtered through a glass microfiber filter GF/F with 0.45 µm of pore size under vacuum. The liquid fractions were kept at 4 °C and the filters were frozen before freeze-drying (-21 °C, 6 mbar) and conserved at room temperature until analysis. This last fraction is considered as the particulate phase. Those thick samples which filtration was not possible were directly frozen and freeze-dried. Once lyophilized, the samples were stored in aluminium containers at room temperature and in darkness until analysis. Information regarding each STP, sampling and conservation conditions are summarized below.

- STP-1.

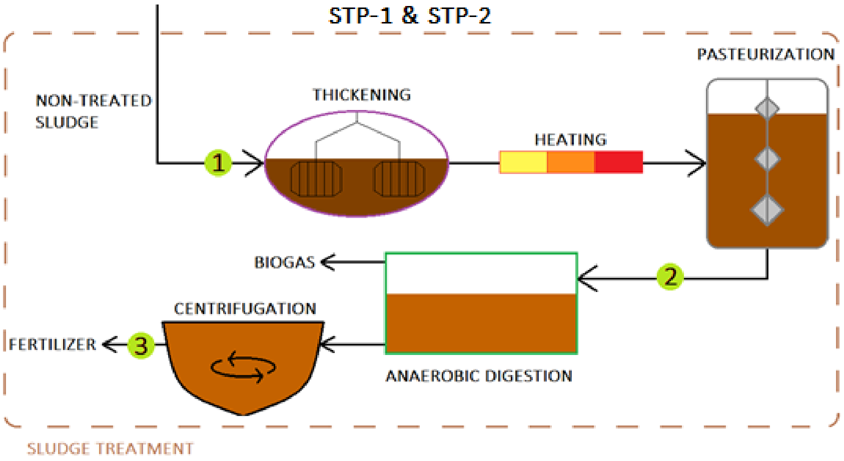


Figure 11. Simplified scheme of the sludge treatment carries out in STP-1 and STP-2. Sampling points are specified with numbers 1, 2 and 3 for raw sludge, post-pasteurized sludge and digested sludge, respectively.

STP-1 receives mainly industrial wastewater (up to 50%) as well as urban wastewater corresponding to 120,000 population equivalents. The influent wastewater undergoes flocculation ($ClFeO_4S$ and polyamine) and sedimentation. First sludge (after a first clarifier) and secondary sludge (after a second clarifier) are mixed. Afterwards, the sludge is thickened and then, follows a progressive heating to start the decomposition and submit a pasteurization treatment (≥ 65 °C) for at least 1h. The resulting product is cooled up to 40 °C and digested under anaerobic and mesophilic conditions for at least 15 days. A reduction of a third of the sludge volume occurs, and biogas is produced, which is collected and used in energy production. After digestion, the sludge is dewatered by centrifugation and the resulting product is used as fertilizer to enrich agricultural soil. A simplified scheme is shown in Figure 11. A 3-days sampling season took place in November 2020. A sample from raw sludge, post-pasteurization sludge, and digested and dewatered sludge was collected each day in glass bottles and kept at 4 °C until analysis. Table 4 summarizes the samples collected from STP-1.

Table 4. Sludge samples from STP-1.

Sludge	Phase	Date	Abbreviation
Raw	Particulate	11.11.2020	R_(1)_P_1
Raw	Dissolved	11.11.2020	R_(1)_L_1
Raw	Particulate	12.11.2020	R_(1)_P_2
Raw	Dissolved	12.11.2020	R_(1)_L_2
Raw	Particulate	13.11.2020	R_(1)_P_3
Raw	Dissolved	13.11.2020	R_(1)_L_3
Post pasteurization	Particulate	11.11.2020	P_(1)_P_1
Post pasteurization	Dissolved	11.11.2020	P_(1)_L_1
Post pasteurization	Particulate	12.11.2020	P_(1)_P_2
Post pasteurization	Dissolved	12.11.2020	P_(1)_L_2
Post pasteurization	Particulate	13.11.2020	P_(1)_P_3
Post pasteurization	Dissolved	13.11.2020	P_(1)_L_3
Digested	Solid	11.11.2020	D_(1)_S_1
Digested	Solid	12.11.2020	D_(1)_S_2
Digested	Solid	13.11.2020	D_(1)_S_3

- STP-2

The same sampling strategy followed for STP-1 was performed in STP-2. Sludge is produced and treated similarly in STP-2 too (Figure 11) with small differences i.e., polyacrylamide is used as flocculant. It has been designed to have a capacity for 170,000 population equivalents. The sludge before and after decomposition reaches around 7,600 kg and 4,900 of dry matter per day, respectively, and 5,000 tonnes of dehydrated sludge is produced per year.

Three samples from each sampling site in the STP-2 were collected in 3 consecutive days in December 2020. One litre of raw sludge (semi-liquid) and post-pasteurized sludge (semi-liquid) were collected in a glass container. Digested and dewatered sludge (semi-solid) was gathered in sterile sample bags. A total of 9 samples were collected from STP-2 and they are summarized in Table 5.

Table 5. Samples from STP-2.

Sludge	Phase	Date	Abbreviation
Raw	Solid	02.12.2020	R_(2)_S_1
Raw	Solid	03.12.2020	R_(2)_S_2
Raw	Solid	04.12.2020	R_(2)_S_3
Post pasteurization	Particulate	02.12.2020	P_(2)_P_1
Post pasteurization	Dissolved	02.12.2020	P_(2)_L_1
Post pasteurization	Particulate	03.12.2020	P_(2)_P_2
Post pasteurization	Dissolved	03.12.2020	P_(2)_L_2
Post pasteurization	Particulate	04.12.2020	P_ (2)_P_3
Post pasteurization	Dissolved	04.12.2020	P_ (2)_L_3
Digested	Solid	02.12.2020	D_ (2)_S_1
Digested	Solid	03.12.2020	D_ (2)_S_2
Digested	Solid	04.12.2020	D_ (2)_S_3

- STP-3

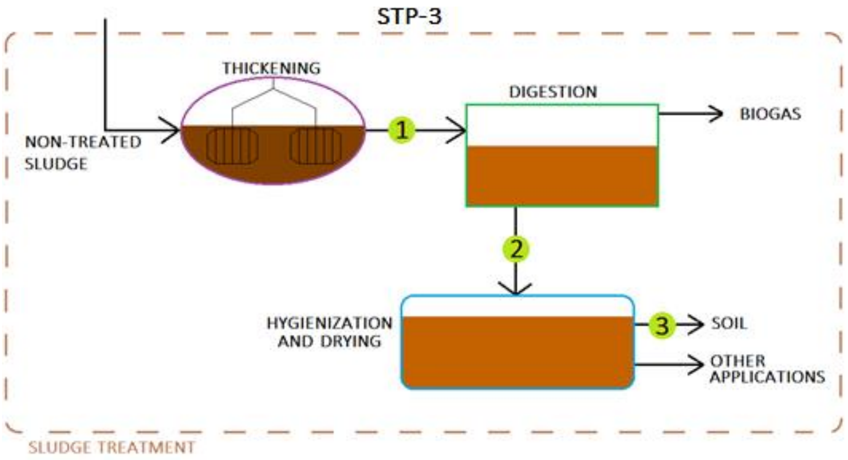


Figure 12. Simplified scheme of the sludge treatment done in STP-3

The third sampled facility, coded as STP-3, was the biggest of all. It treats wastewater equivalent to 750,000 population equivalents. Hence, around 100 million m^3 of influent water is cleaned every year. The influent undergoes a screening, a sand grit and a chemical treatment. In this last step, chemicals are added to bind small particles together into larger particles that sink and form sludge. As shown in Figure 12, sludge is thickened and then, digested for 20 days (or more). Calcium oxide is added to the non-gaseous product of digestion and then, dried under vacuum to produce soil. The latter contains organic matter, phosphorus and nitrogen, as well as the added calcium oxide. Consequently, it is used as fertilizer and soil-improving, among other applications. A total amount of 38,000 tonnes of this soil is produced per year with a 29% of lime in mass.

Three samples were collected before and after digestion in 3 consecutive days in December 2020. A single grab sample was taken after the hygienization and drying process. The STP-3 sampling sites are shown in Figure 12, and Table 6 summarizes the characteristics of the 7 collected samples in this facility.

Table 6. Samples from STP-3.

Sludge	Phase	Date	Abbreviation
Pre-digestion	Solid	08.12.2020	Pre_ (3)_S_1
Pre-digestion	Solid	09.12.2020	Pre_ (3)_S_2
Pre-digestion	Solid	10.12.2020	Pre_ (3)_S_3
Post-digestion	Solid	08.12.2020	D_ (3)_S_1
Post-digestion	Solid	09.12.2020	D_ (3)_S_2
Post-digestion	Solid	10.12.2020	D_ (3)_S_3
Dried	Solid	12.2020	Dry_ (3)_S

2.3 Sample preparation.

The sample preparation protocol used for the analysis of BTHs and BTRs in WEEE and sludge was previously described by Asimakopoulos et al. for the analysis of benzothiazoles and benzotriazoles in sewage sludge samples from a STP located in Greece [10]. Herein, the proposed sample preparation methodology (with small modifications) was also tested for other families of emerging contaminants: parabens, phthalates, PFCs, bisphenols and benzophenones. Method validation has been completed for all the families.

WEEE

A representative sample of 0.1 g was accurately weighed and placed in a 15 mL Eppendorf tube. The sample was spiked with 20 µL of a mixture of ISs (BTR-d_4 and 5-Me-BTR-d_6 in MeOH, 1 µg L^{-1}) and added 5 mL of MeOH:deionized water (1:1) adjusted at pH<3 with HCl. Then, LSE was accomplished by 1 min vortex and ultrasonication (45 min, 35 °C). Then, the resulting suspension was centrifuged (5 min, 4,000 rpm) and the supernatant was transferred into a 50 mL PP tube. The obtained solution was diluted until 50 mL with acidified deionized water (HCl, pH<3) and vortex for 1 min prior the SPE.

Samples were concentrated and cleaned up following a SPE, using Strata X cartridges. Cartridges were activated with 10 mL of MeOH and conditioned with 10 mL of deionized water (pH<3). After the sample loading, cartridges were rinsed with acidified (pH<3, HCl) deionized water water (10 mL) and dried under vacuum. The elution of the compounds was achieved with 10 mL of a mixture of ACN:MeOH (1:1) and collected in 15 mL PP tubes. The obtained extract was concentrated until almost dryness under a gentle nitrogen stream (35 °C) and reconstituted until a final volume of 1 mL MeOH:deionized water. The final extract was transferred to a vial, ready for analysis. In case of BTHs and BTRs the extracts were acidified with formic acid. Thus, an aliquot of the sample (90 µL) was mixed with 1% formic acid (10 µL) prior injection in the LC-MS.

Sludge: dissolved phase

In total, 2 mL of sludge samples were filtered when possible after collection. The liquid extract was transferred into a 50 mL Eppendorf® PP tube and filled up with deionized water. The pH was adjusted to <3 with a few drops of concentrate HCl. Then, it was loaded into the cartridge to perform the SPE cleanup and followed the rest of the protocol as described above for WEEE samples.

Sludge*: solid and particulate matter*

An aliquot of approximately 0.1 g of dried sludge was introduced into a 15 mL Eppendorf® PP tube. In the case of the particulate matter, a filter was accurately weighed before and after freeze-drying (particulate median mass: 44.2 mg). Then, it was transferred into a 15 mL tube and followed the same process performed with WEEE samples.

2.4 Instrumental conditions

Chromatographic separation and mass spectrometry detection of the target analytes was performed by UHPLC-MS/MS with a Acquity UPLC system connected to a Xevo TQS triple quadrupole mass spectrometer, furnished with a Z spray ESI source, both acquired from Waters (Milford, MA, USA). Analytes were separated using a Kinetex C_{18} column (30 x 2.1 mm, 1.3µm, 100Å Phenomenex) serially connected to a Phenomenex C_{18} guard column.

The sample volume automatically injected was 5 µL. Ultrapure water (A) and ACN (B), both acidified with FA 0.1% were used as mobile phases at a constant flow rate of 0.4 µL min^{-1}. The temperature in the column was set at 30 ºC. The mobile phase gradient was programmed as follows: 5% B (0-0.1 min), ramp from 5-100% B (0.1-3 min), 100% B (3.1-4 min), ramp from 100-5% B (4.1-5 min).

Analytes were ionized in positive mode (ESI+). Nitrogen was employed as nebulizing and drying gas in the ionization source (400ºC, 800 L hour^{-1}). The capillary voltage was maintained at 3.0 kV, cone at 25 V and source offset at 30 V. Two transitions were recorded per compound considering a time window of 60 s around their retention time (RT). The dwell time per transition was automatically adjusted by the MassLynx software to obtain 12 points per peak assuming an average baseline peak width of 5 s.

Monoisotopic mass, transitions, collision energies and cone voltages are summarized in Table 7.

Table 7. Monoisotopic mass, precursors and daughter ions for quantification and qualification transitions, collision energies and cone voltage for the LC-MS/MS analysis.

Abbreviature	**Monoisotopic mass[a] (Da)**	**Cone Voltage (V)**	**Q_1 (CE, V)[a]**	**Q_2 (CE, V)[b]**
BTH	135.0143	44	136 → 109 (18)	136 → 65 (22)
2-S-BTH	166.9863	8	168 → 135 (20)	168 → 92 (20)
2-OH-BTH	151.0092	8	152 → 80 (22)	152 → 124 (16)
2-ABTH	150.0252	28	151 → 124 (18)	151 → 109 (20)
2-MeS-BTH	181.0020	26	182 → 167 (22)	182 → 109 (32)
2-M-BTH	220.0670	8	221 → 177 (18)	221 → 109 (30)
2-Cl-BTH	168.9753	26	170 → 134.8 (24)	170 → 109.1 (22)
2-SCNMeS-BTH	237.9693	8	239 → 136 (26)	239 → 180 (14)
2-Me-BTH	149.0299	35	150 → 109.1 (20)	150 → 65.1 (32)
BTR	119.0483	28	120 → 65 (16)	120 → 92 (14)
XTR	147.0797	16	148 → 93 (16)	148 → 77 (24)
BTR-COOH	163.0382	8	164 → 80 (18)	164 → 108 (18)
5-Cl-BTR	153.0094	24	154 → 99 (22)	154 → 74 (24)
1-OH-BTR	135.0433	24	136 → 91 (18)	136 → 119 (14)
5-ABTR	134.0593	42	135 → 107 (16)	135 → 80 (16)
TTR	133.0640	18	134.2 → 79 (18)	134.2 → 77 (16)
BTR-d4	123.0735	38	124 → 69 (18)	124 → 96 (16)
5-Me-BTR-d6		24	140 → 81 (22)	140 → 85 (20)

[a]data from Chemspider.com. [b]CE=Collision Energy, expressed in V.

2.5 Calculations for method validation and quantification and data analysis.

The analytical methodology was validated in terms of its trueness by determining the absolute recoveries (AR%), matrix effects (ME%), method recoveries (MR%) and relative method recovery (RMR%). Hence, a composite sample was prepared for each matrix by mixing a fraction of all the samples of sludges or WEEE. These composite samples were fortified at 3 different levels (5, 10 and 20 µg L^{-1}) of the target analytes and 20 µg L^{-1} of the ISs (n=3 for each fortification level) prior the extraction (spikes pre-extraction) and just before instrumental analysis (spikes post-extraction). A blank sample (only spiked with ISs pre-extraction) was also prepared in duplicate (n=2) for each matrix from the composite samples.

The instrumental dynamic range was evaluated with an external calibration curve for all the TA from 0 to 20 µg L^{-1} (n=11 points) fortified with IS at a level of 20 µg L^{-1} in MeOH:desionized water (1:1) acidified with FA (0.1%).

An internal standard quantification approach was applied making use of two different ISs: BTR-d_4 and 5-Me-BTR-d_6. The assignment of each IS throughout the analytes depended on the RT of each compound. Hence, BTR-d_4 was used to correct the analytes with RT lower than 2.0 minutes (average between both IS RTs) and 5-Me-BTR-d_6 to correct the compounds with RT above 2 minutes.

BTHs and BTRs were quantified by comparing the average peak areas for the spikes pre-extraction (after blank subtraction) with those obtained for the samples (after blank subtraction). All areas were first corrected with the corresponding IS.

- *Absolute recoveries (AR%)*

Absolute recoveries for the extraction procedure were determined by comparing the MRM peak average areas obtained from spiked pre-extraction (A_{sp}) and spikes post-extraction (A_{MM}). Hence, formula (1) was applied:

$$AR\% = \frac{A_{sp}}{A_{MM}} * 100 \tag{1}$$

Calculations were performed in both studied matrices. Values close to 100% indicate a quantitative recovery of the compounds during the extraction, and no analyte loss was produced during the sample treatment. Environmental samples may not always reach high recoveries because of the complexity of the matrix and the analytical protocols they undergo.

- *Matrix effect (ME%)*

The efficiency of the analyte ionization in the ESI source can be compromised by the components of the matrix, which might compete for the ionization. Thus, the obtained response for the same analyte can be different between the standard and the real sample. To calculate this, equation (2) was applied:

$$\mathrm{ME\%} = (\frac{A_{MM} - A_{\mathrm{b}}}{A_{std}} - 1) * 100 \tag{2}$$

where A_b is the average peak area for each compound in the blanks, and A_{std} is the area obtained from direct injections (5 µL) of equivalent amounts of standards in MeOH:desionized water (1:1) (acidified with FA, 0.1%) solutions. Same fortification level was applied for A_{MM} and A_{std}. Values close to 0% indicates the absence of matrix effect. On the other hand, positive values indicate an enhancement and negatives, a suppression of the signal.

- *Method recovery (MR%)*

The method recovery, accounting for percentage of loss area due to the methodology protocol and for the matrix effect, was calculated as the peak area obtained for the spike pre-extraction after the blank subtraction, and divided by the peak area obtained for the same standard concentration in solvent, as shown in equation (3):

$$MR\% = \frac{|A_{sp}| - |A_{\mathrm{b}}|}{A_{std}} * 100 \tag{3}$$

MR% values close to 100 % indicate that no analyte loss was registered during method protocol and the matrix is not affecting the result, therefore, calculation of result might be done with an external calibration.

- *Relative method recoveries (RMR%)*

Relative method recoveries were calculated as show in equation (4):

$$RMR\% = MR\% * {A_{sp,IS}}^{-1} A_{std,IS} \tag{4}$$

where $A_{sp,IS}$ is the peak area for the IS in the spikes pre-extraction and $A_{std,IS}$ is the peak area for the IS in solvent for the same fortification level. Values close to 100% indicate that the assigned IS perform a satisfactory correction of the method deficiencies and the final method trueness is appropriate.

Regarding data analysis, MassLynx and TargetLynx softwares (Waters, Milford, U.S.) were employed for the obtention of data from the LC-MS/MS and data treatment. Excel (Microsoft, 2010) was used to perform data analysis.

2.6 Calculation of distribution coefficients.

The sorption of the different studied compounds onto the sludge was estimated by the calculation of the distribution coefficients (K_d) as shown in the equation (5):

$$K_d = \frac{C_s}{C_w} \tag{5}$$

where C_s is the concentration of the compound in the particulate matter phase and C_w is the concentration of the compound in the dissolved phase. For quantification purposes, detected compounds with values under the LOQ have been substituted by the LOQ/2 [87]. Resulting K_d values indicated the tendency of each compound to adsorb into the solid phase.

2.7 Quality assurance and quality control.

Quality assurance and quality control (QAQC) measures were required to avoid any potential contamination during sample preparation and analysis. Thus, all glass material was cleaned with distilled water (n=3) and rinsed with MeOH (n=3) before use. Any contamination coming from the laboratory or solvents was corrected with procedural blanks submitted to the same procedure as the samples and injected during the analysis of the real samples. All the samples were fortified with a mixture of labelled IS to account for any signal losses during sample preparation and instrumental analysis. Reagent blanks, i.e., blank samples without the matrix, spiked with IS (20 µg L^{-1}), were also performed, and its signal (area) was subtracted from all the samples and blanks, assuming to be contamination and background noise in all samples within the batch.

During the instrumental analysis, an external standard solution at a concentration of 10 µg L^{-1} in MeOH:deionized water followed by a MeOH solvent were analyzed every 15 injections to determine the instrumental repeatability, possible carry over, as well as potential retention time drifting. Internal standards were used as reference during the QAQC tests too.

3. Results and discussion

3.1 UHPLC-MS/MS – Instrumental method validation

Two MRM transitions per compound were recorded. The transition providing the highest response was selected for quantification purposes (Q_1). Nevertheless, the second most intense transition (Q_2) was selected for confirmation. This is usually needed in environmental samples due to the low concentrations and the possible effects of the matrix. The ratio between the area of Q_1 and Q_2 is the ion ratio and is used for confirmation purposes too (Table 8). The first peak which fulfil the requirements of ion ratio and linearity is considered as the instrumental limit of quantification (iLOQ), and instrumental limit of detection (iLOD) is calculated as iLOQ/3. Herein, iLOQs ranged from 0.05 to 5 µg L^{-1} in all TA (Table 8). Such low concentrations are possible due to the high sensitivity associated to UHPLC-ESI-MS/MS, and are absolutely paramount to efficiently determine EPs in environmental samples, as they are, both present and still harmful, at trace levels. Moreover, acidification prior analysis improved the retention in the chromatographic column resulting in better peak shape (Figure S1, Appendix B) and consequently, detection became easier for the lowest concentrations.

Table 8. Instrumental limits of quantification and detection (µg L^{-1}), RT and ion ratio.

Compound	iLOQ (µg L^{-1})	iLOD (µg L^{-1})	RT[a]	Ion ratio[a] (RSD%)
1-OH-BTR	0.2	0.07	0.76	17.3 (5)
2-ABTH	0.1	0.03	0.91	84.5 (4)
2-Cl-BTH	5.0	1.67	2.86	43.1 (12)
2-M-BTH	0.05	0.02	2.90	94.3 (1)
2-Me-BTH	1.0	0.33	2.94	19.3 (29)
2-MeS-BTH	0.5	0.17	3.15	13.7 (29)
2-OH-BTH	2.0	0.67	2.70	92.5 (30)
2-S-BTH	0.2	0.07	2.87	54.1 (7)
2-SCNMeS-BTH	1.0	0.33	3.18	218 (1)
5-ABTR	0.1	0.03	0.29	28.3 (2)
5-Cl-BTR	0.05	0.02	2.67	18.0 (2)
5-Me-BTR-d6	-	-	2.50	127 (2)
BTH	5.0	0.33	2.78	36.7 (30)
BTR	0.1	0.03	1.57	36.6 (4)
BTR-COOH	2.0	0.67	1.18	73.0 (5)
BTR-d4	-	-	1.53	59.1 (4)
TTR	0.05	0.02	2.51	41.8 (2)
XTR	0.1	0.03	2.74	85.9 (1)

[a]Calculated as average at three fortification levels (5, 10, 20 µg L^{-1}) in external calibration curve.

The RT for each compound is presented in Table 8. The ratio between the RT of a compound and the RT of the IS is known as relative retention time (RRT). The RT of a compound may vary during the analysis because of the matrix but also due to the chromatographic parameters. As the RT of the IS should vary in a similar proportion as the RT of the analyte, drifting times will be compensated in the RRT. Ranges for the RRTs ± 0.1 were established in the external calibration curve and in the spikes (before and after extraction). Analytes in samples with RRTs out of that range were considered as non-detected.

Precision was studied though the injection of a mixture of 10 μg L^{-1} standard along the sequence intra-day (repeatability) and inter-day (reproducibility) (Table 9). Linearity of the calibration curve was considered between the iLOQ and the maximum concentration which conserved the linearity. A coefficient of determination (R^2) close to 1 was used to prove the linearity in this range. Excellent linearity was observed with R^2 higher than 0.99 for all analytes except for 2-Cl-BTH, 2-OH-BTH and 2-SCNMeS-BTH (Table 9).

Precision, linear range and R^2 of the calibration curve are shown in Table 9. Linear equations and calibration curves of each compound are shown in Figures S2 – S17 in the Appendix B.

Table 9. Precision and calibration parameters for BTHs and BTRs.

	Precision		**Linear range** [c]	**R^2**
	RSD% Intra-day [a]	RSD% Inter-day [b]	(μg L^{-1})	
1-OH-BTR	17	13	0.2 - 20	0.9968
2-ABTH	17	16	0.1 - 20	0.9952
2-Cl-BTH	19	30	5.0 - 20	0.9745
2-M-BTH	6	26	0.05 - 20	0.9955
2-Me-BTH	15	24	1.00 - 20	0.9905
2-MeS-BTH	11	27	0.5 - 20	0.9933
2-OH-BTH	13	31	2 - 20	0.9859
2-S-BTH	15	26	0.2 - 20	0.9909
2-SCNMeS-BTH	13	23	1 - 20	0.9763
5-ABTR	15	28	0.1 - 20	0.9959
5-Cl-BTR	7	20	0.05 - 20	0.9971
BTH	21	23	5 - 100	0.9919
BTR	2	8	0.1 - 20	0.9967
BTR-COOH	22	16	2.0 - 20	0.9957
TTR	3	23	0.05 - 20	0.9960
XTR	6	16	0.1 - 20	0.9943

[a]N=7. [b]3 days, N=20 except BTH (2 days, N=12). [c]Range considered from iLOQ to the maximum value in the calibration with linearity.

3.2 Overall method validation

Obtained recoveries and matrix effects (except for BTH) are compiled in Table 10 and Table 11, for WEEE and sludge, respectively, at a 20 µg L^{-1} fortification level. Recoveries and matrix effects for the other fortification levels studied are presented in the Appendix A (Table S1-S4). The proposed methodology was suitable for the extraction of 11 analytes out of the 16 studied. Compounds which are not included in Tables 10 and 11 presented high RSD% or the extraction was not good enough. Hence, 2-Cl-BTH, 2-OH-BTH, 2-S-BTH, 2-SCNMeS-BTH and 5-ABTR were proposed to be ruled out of the analytical method in both matrices. AR%, ME%, MR% and RMR% are presented in Tables S5 and S6 for those compounds, as well as a tentative explanation about their lack of performance.

Table 10. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recovery (RMR%) for a fortification level of 20 µg L^{-1} in WEEE samples.

Compound	AR% (RSD%)	ME%	MR% (RSD%)	RMR% (RSD%)
1-OH-BTR	78 (6)	-4	75 (6)	100 (18)
2-ABTH	67 (2)	-31	44 (3)	59 (10)
2-M-BTH	139 (6)	-68	43 (7)	74 (13)
2-Me-BTH	80 (3)	-35	49 (3)	82 (17)
2-MeS-BTH	53 (26)	-7	44 (31)	74 (46)
5-Cl-BTR	104 (1)	-50	52 (1)	69 (12)
BTR	60 (8)	14	63 (9)	84 (21)
BTR-COOH	99 (2)	-25	74 (2)	99 (10)
TTR	66 (3)	-10	57 (3)	77 (15)
XTR	101 (4)	-51	49 (4)	65 (16)

Table 11. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recovery (RMR%) for a fortification level of 20 µg L^{-1} in sewage sludge samples.

Compound	AR% (RSD%)	ME%	MR% (RSD%)	RMR (RSD%)
1-OH-BTR	76 (2)	-4	70 (2)	90 (6)
2-ABTH	75 (2)	-12	65 (2)	84 (6)
2-M-BTH	75 (2)	-53	35 (2)	71 (5)
2-Me-BTH	61 (3)	3	64 (3)	129 (8)
2-MeS-BTH	42 (8)	29	24 (19)	49 (22)
5-Cl-BTR	72 (2)	-42	41 (2)	53 (7)
BTR	78 (3)	90	72 (7)	92 (4)
BTR-COOH	88 (7)	30	105 (8)	135 (2)
TTR	171 (11)	75	116 (29)	149 (31)
XTR	89 (12)	-20	40 (20)	51 (27)

Absolute recoveries remained above 59% for all the compounds with the exception of 2-MeS-BTH, which were below 53% in both matrices (Tables 10 and 11). On the other hand, AR% for 2-M-BTH in WEEE and TTR in sludge were above 100%. Even though some of these values are not in the range of 70-130%, they were really encouraging due to the number of compounds analyzed with the same sample preparation applied to two completely different matrices. Additionally, the lack of accuracy provided for some of the AR% values was overcome with a suitable correction with the ISs.

Regarding ME%, in WEEE, most of the compounds suffered suppression (ME% < 0) during the ionization in the ESI source. Potential matrix effects during the ionization were taking into consideration during the quantification through the preparation of samples spiked before the sample preparation.

The MR% for WEEE and sludge (fortification level: 20 µg L^{-1}) ranged between 40 – 116% except for 2-M-BTH and 2-MeS-BTH in sludge. However, results of MR% showed that external calibration was not an option to quantify the concentration of these compounds (Figure S18). Regarding RMR%, values between 59-100% were obtained in WEEE at a fortification level of 20 µg L^{-1} with excellent RSD% except for 2-MeS-BTH. The range in sludge was wider, again, due to the complexity of the matrix.

Sensitivity of the method was evaluated with the method limit of quantification (mLOQ) and method limit of detection (mLOD), calculated for a nominal mass of 0.1 g (Table 12).

Table 12. Sensitivity of the method (ng g^{-1}).

Compound	mLOQ	mLOD	Compound	mLOQ	mLOD	Compound	mLOQ	mLOD
1-OH-BTR	2.0	0.67	**2-OH-BTH**	20	6.7	**BTH**	50	17
2-ABTH	1.0	0.33	**2-S-BTH**	2.0	0.67	**BTR**	1.0	0.33
2-Cl-BTH	50	17	**2-SCNMeS-BTH**	10	3.3	**BTR-COOH**	20	6.7
2-M-BTH	0.50	0.17	**5-ABTR**	1.0	0.33	**TTR**	0.5	0.17
2-Me-BTH	10	3.3	**5-Cl-BTR**	0.50	0.17	**XTR**	1.0	0.33
2-MeS-BTH	5.0	1.7						

BTH was suspected to be degraded in the stock solutions, as it was not detected in any spiked sample at any fortification level. A newly fresh stock solutions was prepared confirmed it. Additionally, the presence of two peaks in the samples and the absence of spikes in the matrix for BTH aroused the necessity of determinate which one was the peak of BTH. It was proved with the addition of BTH just before the analysis, in a composite sample which has undergone the complete protocol, at 3 fortification levels and its analysis (Figure S19).

The diverse matrix and families of EPs made a complicated task to extract all the analytes and reduce the matrix effect with a same methodology. However, this method showed acceptable recoveries and matrix effects for a high number of compounds.

3.3 Occurrence of BTRs and BTHs in WEEE

Fifteen WEEE samples were analyzed, and the concentrations determined for both families, BTRs and BTHs, are shown in Table 13. BTR and TTR were found in all the samples with concentrations ranged between 2.5 – 410 ng g^{-1} and <LOQ - 46 ng g^{-1}, respectively. Regarding the BTHs, the detection rate (DR%) for 2-M-BTH and BTH reached a 100% and BTH was the one found at highest concentration ranging between 85 and 8,831 ng g^{-1} (Table 13). Nevertheless, 2-M-BTH presented lower concentrations (median: 4.4 ng g^{-1}). 2-ABTH was found at concentrations between 1.0 and 429 ng g^{-1} in all samples except in Q3. On the other hand, BTR-COOH was detected in only 1 out of 15 samples, and under the limit of quantification.

Regarding samples coded as R1-R9, which were collected in the same facility, R8 presented the highest concentrations of BTRs (ΣBTRs = 496 ng g^{-1}) whose main contributor was BTR with a concentration of 302 ng g^{-1} (Table 13). Nonetheless, all the target compounds, apart from BTR-COOH and 2-MeS-BTH, were identified in this sample. On the other hand, the highest concentrations of BTHs in these samples were found in R3 and R7, presenting ΣBTHs > 1700 ng g^{-1}. It is worth pointing out that the concentration of BTH was remarkably high in all the studied samples. This was probably due to the high production and use of this chemical during the production of WEEE. In addition, BTH is also a common by-product of many other BTHs non studied in this study, such as 2-OH-BTH. Samples R1, R2 and R3 were constituent of electronic equipment with domestic appliances. The three samples showed similarities in the pattern of detected BTRs and BTHs. However, BTH was quantified in R3 at a higher concentration. Sample R7 was the reject fraction carried out before the plastic separation in R1, R2 and R3, and generally presented a higher content of ΣBTRs and of ΣBTHs than in those, except for R3 due to its high content of BTH. A similar comparison may be done for R4, R5, R6 and its rejected fraction, i.e., R8. In this case, similar occurrence of ΣBTHs was found for all these samples. Nevertheless, the content of BTRs in R8 was considerably higher.

In regard to Q samples, Q3 and Q4 came from bottles and cap bottles, respectively, and the occurrence of BTRs and BTHs were predominant in the caps. Pyrolysis was suspected to reduce the content of 2-M-BTH, 2-MeS-BTH, BTH, BTR and TTR, as their concentrations were clearly higher in Q3 y Q4 than the ones found in Q2, which was the char resulting of the pyrolytic treatment of a mixture of Q3 and Q4 (9:1). In fact, based on those concentrations, removal efficiencies (RE%) ranging between 84 – 100%, could be attributed to this treatment (Table 13). These RE% were calculated as the difference of concentrations between the samples before pyrolysis and after pyrolysis, divided by the concentrations before pyrolysis. Other compounds such as 1-OH-BTR, BTR-COOH and XTR were non-detected in these samples. In fact, the reduction of the components in the char may be explained with an analysis of the aqueous and oil phase produced after condensation of the gas phase formed during the pyrolysis. Due to the polarity of the compounds, aqueous phase is susceptible to dissolve the analytes. Results evidenced the efficiency of the pyrolysis due to the reduction of these EPs in WEEE so its applicability may increase in the concerned industries.

Generally, Q5 and Q6 presented similar concentration pattern concentrations with BTR, XTR and 2-M-BTH, standing out among the others. Both were principally food waste bags, which explains their similarity.

In comparison to other studies published in the scientific literature, the occurrence of BTRs and BTHs observed in the present study was lower than the one reported by Wenzheng et Al. in indoor dust samples from an e-waste dismantling area in Qingyuan (China) [41] . However, XTR was founded at higher concentrations, and the occurrence of 1-OH-BTR was low in both studies, with concentrations generally under the LOQ. To the best of our knowledge, no data has been previously reported for the presence of BTHs and BTRs in WEEE samples.

Table 13. Obtained concentrations of BTRs and BTHs in WEEE samples (ng g^{-1}).

Sample	1-OH-BTR	5-Cl-BTR	BTR	BTR-COOH	TTR	XTR	ΣBTRs [e]	2-ABTH	2-M-BTH	2-Me-BTH	2-MeS-BTH	BTH	ΣBTHs [g]
R1	<LOQ [d]	2.9	30	n.d.	4.1	n.d.	37	8.9	18	n.d.	n.d.	792	818.9
R2	<LOQ	0.6	16	n.d.	3.0	n.d.	19.6	7.7	4.4	n.d.	12	520	544.1
R3	n.d. [e]	1.9	25	n.d.	5.3	n.d.	32.2	28	8.7	n.d.	n.d.	2,819	2,855.7
R4	n.d.	0.5	3.8	n.d.	3.6	<LOQ	7.9	1.0	2.2	n.d.	n.d.	140	143.2
R5	n.d.	n.d.	6.3	n.d.	1.5	n.d.	7.8	6.2	2.2	n.d.	26	170	204.4
R6	n.d.	0.6	3.2	n.d.	<LOQ	n.d.	3.8	3.0	0.7	n.d.	n.d.	131	134.7
R7	n.d.	7.9	84	n.d.	15	9.2	116.1	13	60	38	289	1,333	1,733
R8	155	1.0	302	n.d.	2.6	35	495.6	7.5	55	22	n.d.	119	203.5
R9	n.d.	<LOQ	33	n.d.	19	17	69	3.5	0.7	29	25	317	375.2
Q1	<LOQ	n.d.	20	n.d.	18	n.d.	38	3.1	<LOQ	n.d.	8.0	91	102.1
Q2	n.d.	n.d.	2.5	n.d.	1.7	n.d.	4.2	3.5	<LOQ	16	<LOQ	85	104.5
Q3	n.d.	n.d.	4.1	n.d.	7.3	n.d.	11.4	n.d.	41	22	6.3	120	189.3
Q4	n.d.	8.6	123	n.d.	46	n.d.	177.6	429	21	n.d.	414	8,831	9,695
Q5	n.d.	<LOQ	338	<LOQ	7.6	9.9	355.5	5.7	3.4	n.d.	90	377	476.1
Q6	<LOQ	1.4	410	n.d.	8.7	10	430.1	6.5	3.4	n.d.	286	2,502	2,797.9
Median [a]	- [f]	1.4	25	-	6.3	10	37	6.4	4.4	22	26	317	375
D.R.% [b]	33	73	100	6.7	100	40	100	93	100	33	67	100	100
RE% [c]	n.d.	100	84	n.d.	85	n.d.	85	92	100	19	100	91	91

[a] Calculated for values above the LOQ. [b] Detection Rate. [c] Removal efficiencies for the pyrolysis. [d] Under the limit of quantification. [d] Non-detected. [e] Total concentration of BTRs in sample. [f] Non-calculated. [g] Total concentration of BTRs in sample.

3.4 Occurrence of BTHs and BTRs in sewage sludge.

A previous study has reported the differences between the concentrations of BTRs and BTHs in primary sludge (after a first sedimentation) and secondary sludge (after a secondary sedimentation) showing the efficiency of the associated biological STP treatment [23]. Other study included the occurrence of these compounds along the sludge treatment [75]. However, data is generally scarce for BTHs and BTRs. The present study focused on the occurrence and evolution of these EPs along the sewage sludge treatment in 3 different STPs. Hence, a total of 25 sewage sludge samples from 3 different STPs in Norway were studied. Nine of these samples were first filtered so the occurrence of BTRs and BTHs was reported in dissolve and particulate phase. Obtained data is presented in Tables 14-16 for STP-1, STP-2 and STP-3, respectively.

TTR and XTR have been detected in all the sewage sludge samples, and they contribute to a 39%, 51% and 87% of the total mass of BTRs in STP-1, STP-2 and STP-3, respectively. However, the contribution of BTR also reached a high percentage in STP-1 and STP-2 (56% and 48%, respectively) even if it presented lower detection rates (56% and 11%, respectively). A lower contribution for the total mass of BTRs was found in STP-3 (10%), despite BTR was always detected. On the other hand, BTR-COOH has been only detected in some samples and under the limit of quantification. The polarity and low LogP of this compound suggested a trend to dissolve into water, which may explain its low occurrence in sewage sludge. The contribution of 1-OH-BTR and 5-Cl-BTR was low, under a 5%, in all the STPs.

2-MeS-BTH has been the main compound in the family of BTHs in terms of detection rate, and along with BTH, they showed the highest concentrations in all the studied sewage sludge samples. Hence, 2-MeS-BTH contributed to the total BTHs mass with 66%, 47% and 52% for STP-1, STP-2 and STP-3, respectively. Even though BTH was not ubiquitous (18 of 25 samples), its contribution to the total BTHs mass reached similar levels than 2-MeS-BTH (49% and 46%) in STP-2 and STP-3. 2-ABTH was detected in 17 of 25 samples. However, only 7 samples presented values above the LOQ, and the concentrations were generally low. In previous studies, 2-ABTH has also been poorly detected in sludge samples and low concentrations were reported [10][23]. 2-M-BTH and 2-Me-BTH were present in lower concentrations despite their detection rates, in particular, 18/25 and 17/25, respectively.

Table 14. Estimated concentrations of BTRs and BTHs in sewage sludge samples from STP-1. Results are given in ng L^{-1} of wet sludge (w) for liquid phase and particulate matter, and in ng g^{-1} of sludge (dry weight – dw) for solid samples.

Samples	**Units**	**1-OH-BTR**	**5-Cl-BTR**	**BTR**	**BTR-COOH**	**TTR**	**XTR**	**ΣBTRs** [g]	**2-ABTH**	**2-M-BTH**	**2-Me-BTH**	**2-MeS-BTH**	**BTH**	**ΣBTHs** [h]
R_(1)_L_1	ng L^{-1} (w) [c]	n.d. [e]	<LOQ [f]	n.d.	n.d.	115	276	391	n.d. [d]	n.d.	n.d.	n.d.	<LOQ	<LOQ
R_(1)_L_2		148	n.d.	n.d.	n.d.	188	2,860	3,196	n.d.	49	n.d.	n.d.	<LOQ	49
R_(1)_L_3		n.d.	n.d.	n.d.	n.d.	75	1,988	2,063	n.d.	<LOQ	n.d.	n.d.	n.d.	<LOQ
R_(1)_P_1	ng L^{-1} (w)	n.d.	<LOQ	n.d.	n.d.	n.d.	816	816	<LOQ	114	n.d.	773	<LOQ	887
R_(1)_P_2		2,153	91	239	n.d.	77	12,004	14,564	61	227	n.d.	6,399	22,894	29,581
R_(1)_P_3		7,447	<LOQ	152,658	n.d.	7,627	9,468	177,200	<LOQ	156	n.d.	4,768	n.d.	4,924
P_(1)_L_1	ng L^{-1} (w)	n.d.	<LOQ	n.d.	n.d.	4,741	3,194	7,935	<LOQ	n.d.	n.d.	n.d.	n.d.	<LOQ
P_(1)_L_2		n.d.	n.d.	n.d.	n.d.	432	1,931	2,363	<LOQ	n.d.	n.d.	n.d.	n.d.	<LOQ
P_(1)_L_3		n.d.	n.d.	n.d.	n.d.	1,028	2,524	3,552	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P_(1)_P_1	ng L^{-1} (w)	n.d.	78	16,313	<LOQ	18,17	2,302	36,863	n.d.	156	1,239	5,287	17,081	23,763
P_(1)_P_2		n.d.	107	3,837	n.d.	5,616	1,31	10,87	n.d.	<LOQ	1,151	2,767	12,758	16,676
P_(1)_P_3		321	44	5,009	n.d.	7,137	1,66	14,171	n.d.	n.d.	1,340	4,217	15,430	20,987
D_(1)_S_1	ng g^{-1} (dw) [d]	n.d.	1.3	n.d.	<LOQ	11	5.9	18.2	1.8	1.0	n.d.	131	<LOQ	133.8
D_(1)_S_2		2.5	1.6	n.d.	<LOQ	26	3.1	33.2	1.8	1.2	<LOQ	125	93	221.0
D_(1)_S_3		<LOQ	0.9	n.d.	n.d.	8.0	2.8	11.7	1.3	0.6	<LOQ	119	n.d.	120.9
D.R.% [a]		56	100	56	33	100	100	100	89	89	56	100	78	100
% mass [b]		4	1	56	0	24	15		1	1	1	66	31	

[a]Detection rate. [b]Mass percentage [c]Wet sludge. [d]Dry sludge. [e]Non-detected. [f]Under the limit of quantification. [g]Total concentration of BTRs in sample. [h]Total concentration of BTHs in sample.

Table 15. Estimated concentrations of BTRs and BTHs in sewage sludge samples from STP-2. Results are given in ng L^{-1} of wet sludge (w) for liquid phase and particulate matter, and in ng g^{-1} of sludge (dry weight – dw) for solid samples.

Samples	Units	1-OH-BTR	5-Cl-BTR	BTR	BTR-COOH	TTR	XTR	ΣBTRs [g]	2-ABTH	2-M-BTH	2-Me-BTH	2-MeS-BTH	BTH	ΣBTHs [h]
R_(2)_S_1	ng g^{-1} (dw) [c]	12	<LOQ	n.d.	<LOQ	226	382	620	n.d. [d]	2.1	<LOQ	106	61	169.1
R_(2)_S_2		7.2	1.0	n.d.	n.d.	114	191	313.2	1.4	<LOQ	16	117	82	216.4
R_(2)_S_3		<LOQ [e]	2.0	2,233	n.d.	68	120	2,423	<LOQ [e]	3.2	29	134	488	654.2
P_(2)_L_1	ng L^{-1} (w) [d]	n.d. [f]	31	n.d.	<LOQ	497	515	1,043	n.d.	107	981	n.d.	7,985	9,073
P_(2)_L_2		n.d.	66	n.d.	<LOQ	504	586	1,156	<LOQ	83	n.d.	n.d.	17,539	17,622
P_(2)_L_3		<LOQ	<LOQ	n.d.	<LOQ	1,671	870	2,541	n.d.	52	n.d.	n.d.	5,190	5,242
P_(2)_P_1	ng L^{-1} (w)	1,570	150	n.d.	n.d.	636	1,054	3,410	n.d.	250	1,379	5,823	n.d.	7,452
P_(2)_P_2		1,216	183	n.d.	n.d.	951	1,259	3,609	n.d.	201	1,493	6,273	n.d.	7,967
P_(2)_P_3		179	71	n.d.	n.d.	2,455	2,459	5,164	<LOQ	309	1,489	5,485	n.d.	7,283
D_(2)_S_1	ng g^{-1} (dw)	n.d.	3.6	n.d.	n.d.	122	32	157.6	<LOQ	<LOQ	n.d.	64	n.d.	64
D_(2)_S_2		n.d.	2.6	n.d.	n.d.	316	40	358.6	2.6	0.6	n.d.	58	n.d.	61.2
D_(2)_S_3		n.d.	2.9	n.d.	n.d.	331	57	390.9	1.1	0.7	n.d.	71	n.d.	72.8
D.R.% [a]		67	100	11	44	100	100	100	78	100	67	100	67	100
% mass [b]		0.6	0.4	48	0	32	19		0.4	0.6	4	47	49	

[a]Detection rate. [b]Mass percentage. [c]Dry sludge. [d]Wet sludge. [e]Under the limit of quantification. [f]Non-detected. [g]Total concentration of BTRs in sample. [h]Total concentration of BTHs in sample.

Table 14. Estimate concentrations of BTRs and BTHs in sewage sludge samples from STP-3. Results are given in ng g-1 of sludge (dry weight – dw).

Samples	Units	1-OH-BTR	5-Cl-BTR	BTR	BTR-COOH	TTR	XTR	ΣBTRs [f]	2-ABTH	2-M-BTH	2-Me-BTH	2-MeS-BTH	BTH	ΣBTHs [g]
Pre_(3)_S_1	ng g^{-1} (dw) [c]	46	n.d.	116	n.d.	190	1,073	1425	n.d. [b]	n.d.	12	308	149	469
Pre_(3)_S_2		n.d. [d]	1.8	110	<LOQ	301	1,208	1620.8	2.4	n.d.	<LOQ	254	165	421.4
Pre_(3)_S_3		30	n.d.	39	<LOQ	70	123	262	n.d.	n.d.	<LOQ	229	n.d.	229
D_(3)_S_1	ng g^{-1} (dw)	<LOQ [e]	5.5	57	<LOQ	373	26	461.5	n.d.	n.d.	<LOQ	30	170	200
D_(3)_S_2		3.7	5.4	63	<LOQ	356	37	465.1	1.5	n.d.	<LOQ	34	195	230.5
D_(3)_S_3		<LOQ	4.9	50	<LOQ	243	26	323.4	n.d.	n.d.	13	29	210	252
Dry_(3)_S	ng g^{-1} (dw)	n.d.	6.1	45	n.d.	5.3	4.3	60.7	<LOQ[c]	6.5	n.d.	127	n.d.	133.5
D.R.% [a]		71	71	100	71	100	100	100	43	14	86	100	71	100
% mass [b]		2	1	10	0	33	54		0.3	0.4	1.3	52	46	

[a]Detection rate. [b]Mass percentage. [c]Dry sludge. [d]Non-detected. [e]Under the limit of quantification. [f]Total concentration of BTRs in sample. [g]Total concentration of BTHs in sample.

3.5 Evolution of BTRs and BTHs in sewage sludge treatment.

As indicated in section 2.2, a chemical and biological treatment was applied in all 3 STPs, and they were followed by a pasteurization and a digestion in STP-1 and STP-2. In STP-3 a treatment with lime was also included. Grab samples were collected at different sites along the sewage treatment in every tested STP during 3 consecutive days. Digested sludge in STP-3 was sampled only once. Consequently, the evolution of the EPs concentrations can be evaluated along the treatment. However, the occurrence of EPs might vary day-to-day within the same site because of technical factors, such as industrial discharges, climate conditions, variation in the treatment efficiency, etc. The behavior for each compound in the three STPs is presented in Figures S20-S49.

- **Evolution of BTRs.**

1-OH-BTR was detected in several samples in all monitored STPs. However, its concentration did not present a clear trend (Figures S20-S22). Regardless, it was non-detected in the final sludge in STP-2 and STP-3 (Tables 16 and 17). Some transformation products (TPs) of this compound have been proposed by Katarzynna Kowalska et al. In particular, dihydroxylated benzotriazoles or even the cleavage of the benzene ring was suggested as a biotransformation of 1-OH-BTR [88].

In Figures S23, S24 and S25, the evolution of **5-Cl-BTR** in the different STPs is presented. The concentration of 5-Cl-BTR increased in STP-1 and STP-2 along the treatment. This trend reflected the similarity in the anaerobic digestions in both STPs. Previously, You-Sheng et al. reported concentrations between 65 and 103 ng g^{-1} for primary and secondary sludge and 68 to 114 ng g^{-1} in sludge after an anaerobic digestion so no significant removal or even analyte accumulation was observed after digestion [75]. In STP-3, no specific trend was observed as concentration remain within the same order of magnitude along the whole treatment. Previous studies have reported a 71% of biodegradation of 5-Cl-BTR after 91 days under anaerobic conditions, and BTR was suggested as a transformation product (TP) [89]. In contrast, opposite tendency was observed in real samples after anaerobic digestion where the concentration of 5-Cl-BTR increased. Nonetheless, some technical conditions, such as initial 5-Cl-BTR concentration, treatment duration, among others, were not comparable. In any case, available data is still scarce for this compound and more research is needed to completely understand its behavior during the treatments of sludge in the STPs.

BTR (Figures S26 – S28) was just detected in one out of three raw sludge samples in both STPs, which reflected the variation within same-tank grab samples along the time. However, when detected, concentrations were at high concentrations. In particular, 4,881 ng g^{-1} (Table S7) and 2,333 ng g^{-1} (Table 14) in STP-1 and STP-2, respectively, which may be a consequence of a specific high discharge of this compound around those days, for instance, from industries located in the vicinity of those STPs. However, after the pasteurization, BTR was not detected in STP-2, but detected in STP-1 in the post-pasteurization samples collected in the 3 consecutive days, illustrating the consistency in the corresponding process. Regarding STP-3, values achieved along the process ranged between 39 and 116 ng g^{-1} (Table 16). Concentrations previously reported in solid sludge samples for BTR were in the range of 81 – 219 ng g^{-1} [10][23][75]. Regarding the removal efficiency in STP-3, a slight decrease was observed after the pasteurization step (from 110 to 57 ng g^{-1}, median concentration) whereas in dried soil, the presence of BTR was reduced up to 45 ng g^{-1}. This reduction could be a consequence of the derived dilution during the addition of lime (around a 29% of the total mass in the soil). The effect of the resulting pH in the analyte degradation cannot be discarded either. Aerobic conditions were suggested to be the predominant biodegradation conditions for BTR [89]. This could explain the differences between the high concentration in the raw sludge from STP-3, which was solely made of primary sludge, versus the lower concentrations observed in the raw sludges from STP-1 and STP-2, which were made of a mixture of primary and secondary sludge, and the latter was coming from the STP aeration tanks where the biological secondary treatments took place.

TTR, considered as the mixture of two methylated BTRs (i.e., 4-TTR and 5-TTR), showed differences among the different treatments (Figures S29 – S31). In STP-2, an increment was observed along the process, while in STP-3, the concentration in the final soil was lower, and a removal efficiency (RE%) around 96% was achieved. These RE% were calculated considering the initial and final sludge, according to the data present in Figures S20 – S49. Similarly to TTR, the RE% for **XTR** was high (99%) in STP-3. The digestion produced in STP-3 reduced the majority of the XTR content, and the concentrations after this treatment were uniform. Additionally, Sebastian et al. identified the transformation of 5-Me-BTR to BTR-COOH in batch experiments under oxic conditions [90]. However, **BTR-COOH** was just detected under the limit of quantification in the present study. The polarity of the BTR-COOH together with its low LogP could be consistent with a high tendency to stay in the liquid phase versus being adsorbed onto the sludge during the wastewater treatment.

- **Evolution of BTHs.**

2-ABTH and 2-M-BTH were both detected in 18 out of 25 samples but the concentrations were generally low (Figures S35 – S40). For 2-ABTH, only 9 samples were above the LOQ and measured concentrations remained below 3 ng g^{-1}. As far as we know, no previous studies reported concentrations of 2-M-BTH in sewage sludge samples. Additionally, just a few studies analyzed 2-ABTH in this matrix, and a low detection rate was observed nonetheless, with values under (or close) to the limit of quantification [10][23].

2-MeS-BTH concentrations were remarkably high in the sludge samples at the end of the process line for all STPs (Figures S44 – S46). In fact, differences were observed between the sludge after the pasteurized step (concentrations around 7,000 ng L^{-1}) and after the digestion step (concentrations above 80,000 ng L^{-1}) in STP-1. A similar trend was observed in STP-2 but lower concentrations were reached in the digested sludge. Thus, when comparing the raw sludge with the digested sludge, similar concentrations in STP-2 were found for this compound while in STP-1, the concentrations in the raw sludge were lower. Overall, higher concentrations of 2-MeS-BTH have been found in the present study in comparison to previous reports in the literature for sludge samples [10][23]. Some studies have proposed 2-MeS-BTH as a recalcitrant substance, which would explain its persistence to treatment [20][91].

In contrast, 2-Me-BTH was not detected in the final sludge. This demonstrated the high efficiency of all the STPs in the removal of this compound. However, it should be pointed out that the initial concentrations in the raw sludge were generally low. Data about its environmental occurrence is scarce. In fact, to the best of our knowledge, this is the first study which analyzes 2-Me-BTH in this type of samples.

BTH was not found in the final sludges except for one day in STP-1 (Figures S47 – S49). In STP-3, similar concentrations were determined before and after digestion (median concentration: 149 and 195 ng g^{-1}, respectively) . However, it was completely removed (or transformed) in the final soil. In raw sludge, BTH was found at concentrations ranging from n.d. to 488 ng g^{-1} (Tables 14 – 16). This wide range may be explained because this compound can be a by-product from the degradation of other BTHs present during the process [11][20]. In previous studies, concentrations of BTH have been reported between n.d. – 288 ng g^{-1} (sludge, dry weight) [10][23] while in the present study, concentrations reached 1,104 ng g^{-1} (pasteurized particulate sludge, dry weight) (Table S7).

- **Overall evolution.**

The chemical transformation of BTRs has been described through different pathways including hydroxylation (i.e., 1-OH-BTR, 5-hydroxy-benzotriazole, 4-hydroxibenzotriazole), methylation (i.e., TTR, XTR, 1-Me-BTR) as well as other by-products depending on the conditions [10][11][88][90]. Similarly, BTHs have the trend to transform between them as well as to other compounds. Biodegradation studies found that 2-SCNMeS-BTH could be transformed into 2-S-BTH, which after being exposed to methylation may form 2-MeS-BTH, which is recalcitrant [20]. Additionally, BTH is usually bio-transformed via hydroxylation, and 2-OH-BTH is usually obtained, among other species [20][88]. However, limited information is available about the transformation of these compounds.

The potential transformation of BTRs and BTHs and its TPs make the assessment of their evolution a real challenge. However, the determination of the total concentration of BTRs and BTHs found in each sampled site along the sludge treatment provided an overall insight of their trend. The sum of the concentrations for each step in each STP is presented in Figure 13.

Generally, the total concentration of BTRs decreased along the treatment in all STPs (Figure 13). However, a slight increase of BTHs was noticed in STP-1, while a decrease was observed in STP-2. These differences might be attributed to the different treatment applying, where a production of BTHs as by-product might be taking place. After pasteurization, the total amount of BTRs and BTHs were comparable, which enlightened the homogeneity of this process in each STP. In the case of STP-3, a similar tendency was observed for both families of compounds, which were reduced along the process. This may demonstrate the high removal efficiency of this STP. However, it was only considered the 11 compounds whose occurrence is discussed in this study. Other compounds (e.g., 2-OH-BTH, 2-S-BTH) may be present in the samples and its transformations were not measured, being the cause of the BTH increase during the treatment

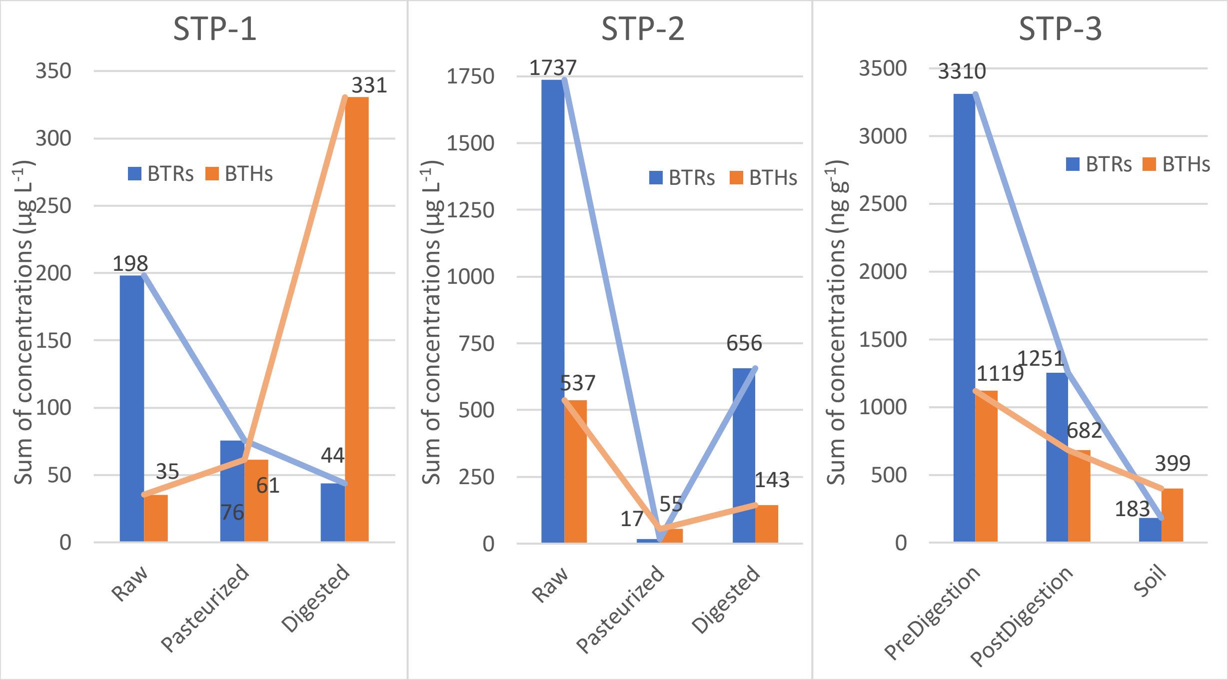


Figure 13. Evolution of the total concentrations of BTRs (blue) and BTHs (orange) in the sludge along each STP treatment. Concentration expressed in ng L^{-1} for STP-1 and STP-2, and ng g^{-1} for STP-3.

3.6 Mass loading of BTHs and BTRs.

Annually, high amounts of treated sludge from STPs are discharged into landfills or applied to soils with agricultural purposes. BTHs and BTRs mass loads per year have been estimated according to the concentrations determined in the present study and the sludge production data provided from the STPs.

Hence, STP-1 produces 15 m^3 of final sludge every day, which supposes around 58 Kg of BTRs and 493 Kg of BTHs every year. The individual contribution to those loads is distributed as 41 Kg y^{-1} of TTR, 12 Kg y^{-1} of XTR, 5 Kg y^{-1} of 5-Cl-BTR, 7 Kg y^{-1} of 2-ABTH, 4 Kg y^{-1} of 2-M-BTH, and 482 Kg y^{-1} of 2-MeS-BTH. Likewise, STP-2 yields the highest amount of BTHs with 2-MeS-BTH (326 Kg y^{-1}) followed distantly by 2-ABTH (6 Kg y^{-1}) and 2-M-BTH (3 Kg y^{-1}), respectively. Even if the treated amount of sewage sludge is larger in STP-2, its BTHs load was slightly lower than in STP-1. However, the total amount of BTRs delivered through the STP-2 final sludge was estimated to be widely bigger, reaching 1,831 Kg per year. TTR contributed with 88% while XTR and 5-Cl-BTR did it with 11% and 1% of the total BTRs mass load, respectively.

STP-3 treats the largest amount of sludge in comparation to the others STPs, and produces 38,000 tons of soil per year with agricultural purposes. Thus, 2,313 Kg of BTRs and 5,056 Kg of BTHs are discharged in the environment every year with this soil. In comparison with other

STPs, the mass load of BTRs was slightly higher than in STP-2 but there is a notorious increment in the total BTHs. Additionally, BTR is the compound which contributes the most to the BTRs mass load, with 1,718 Kg per year. In contract, this compound was not detected at the final sludge in the other STPs. TTR (200 Kg y^{-1}), XTR (164 Kg ye^{-1}) and 5-Cl-BTR (231 Kg y^{-1}) were the other BTRs contributing to final soil load mass. Regarding BTHs, 2-MeS-BTH contributed with a 95% to the BTHs mass abundance in the soil, and the rest corresponded to 2-M-BTH.

3.7 Sludge-water distribution coefficients (K_d).

The distribution of the target analytes between the sewage sludge solid and liquid phases was estimated when possible by calculating their K_d, which are summarized in Table 17. Hence, results of particulate phase were expressed as a mean of all three sampling days in ng g^{-1} (Table S7) and similar for results of dissolved phase (Tables 14 and 15) but in ng mL^{-1}. Resulting K_d were compared to those previously reported on the literature. Similar to other studies [10][23][27], results were above 1. This fact indicates a general trend of distribution onto particulate matter for BTRs and BTHs. It should be noticed that despite the sampling protocol was carried out in 3 consecutive days, concentrations of each compound could widely vary among them due to, for instance, spikes in the industry discharges.

TTR and XTR have been detected in almost all the studied samples. Calculated K_d values in raw sludge were significantly higher than those obtained in the pasteurized sludge. Aikaterini et al. reported K_d values in activated sludge lower than the ones found in the present study [27]. In base to these results, TTR and XTR seem to have a tendency to be adsorbed onto the particulate phase.

To the best of our knowledge, this is the first study reporting estimated K_d values for 1-OH-BTR, 5-Cl-BTR and 2-M-BTH in sewage sludge samples (Table 17). These compounds have the trend to be adsorbed onto the particulate phase. However, concentrations were generally low, and more research is needed to clarify this fact.

2-MeS-BTH has been found in particulate matter with an average concentration of 207 ng g^{-1} for in STP-1 and STP-2 (Table S7). In addition, it was not present in the liquid phase. This is consistent to the fact that it presents the highest logP of all the studied BTHs and BTRs (Table 1). Similar results were reported by Asimakopoulos et al. in secondary sludge, indicating 2-MeS-BTH as the compound with the highest K_d, being absence in the dissolved phase too [10].

Additionally, Athanasios et al. also pointed 2-MeS-BTH as providing the highest K_d among all the BTHs and BTRs in common between their and the present study. [23].

In STP-1, BTR was not found in the dissolved phase and BTH was mainly distributed in particulate phase. In STP-2, BTR was not-detected in either the particulate or dissolve phases and BTH was found principally in the dissolve phase. As each STP received influent wastewater from different areas, the organic matter content might differ between them. Organic matter content is related with the adsorption of other organic compounds onto it [92]. This could explain the wide disparity observed among the BTH K_d values in both STPs.

However, data about the sludge-water distribution for BTRs and BTHs is still limited, and more research is needed.

Table 15. Distribution coefficient values, K_d (in L Kg^{-1}) for BTRs and BTHs in STP-1 and STP-2 samples. K_d is only applied for those samples that had been initially filtered i.e., raw sludge in STP-1 (Raw STP-1), and pasteurized sludge in STP-1 (Past. STP-1) and in STP-2 (Past. STP-2). Concentration between LOQ and LOD were considered as LOQ/2 for K_d calculation purposes.

Samples	1-OH-BTR	5-Cl-BTR	BTR	TTR	XTR	2-M-BTH	2- MeS-BTH	BTH	Reference
Raw STP-1	328	241	P	1809	331 ±332	260	P	337	Present study
Past. STP-1	P [a]	332	P	616 ±554	48 ±16	P	P	P	Present study
Past. STP-2	141	181 ±69	N.C	64 ±6	23 ±23	152 ± 94	P	L [d]	Present study
Activated sludge	N.C. [b]	N.C.	220 ±9	170 ±48 [c]	87 ±17	N.C.	N.C.	N.C.	[27]
Primary sludge	N.C.	N.C.	3.8	2.2	N.C.	N.C.	19	2.1	[10]
Secondary sludge	N.C.	N.C.	288	7.4	N.C.	N.C.	N.C.	148	[10]
Primary sludge	N.C.	N.C.	7 ±3	0.8 ±0.2	N.C.	N.C.	21 ±3	3 ±2	[23]
Secondary sludge	N.C.	-	133 ±104	6 ±4			181 ±56	147 ±63	[23]

[a]Only detected in particulate phase. [b]Not calculated. [c]Considering the 4-methyl-benzotriazole. [d]Only detected in dissolve phase.

4. Conclusions

A versatile and efficient methodology has been proposed for the analysis of more than 40 compounds from different families of EPs (parabens, phthalates, PFCs, bisphenols, benzophenones, BTRs and BTHs) in several complex solid environmental matrices. Focusing on benzotriazoles and benzothiazoles, the proposed methodology has been satisfactorily validated for the analysis of 11 compounds in WEEE and sewage sludge samples.

Additionally, the occurrence of BTRs and BTHs in WEEE has been studied in 15 samples. To the best of our knowledge, this is the first study which focused on the analysis of these EPs in WEEE. Obtained concentrations showed a predominance of BTR and BTH which were present in all the samples with median concentrations of 25 and 317 ng g^{-1}, respectively. Additionally, the thermal treatment conducted in Q2 demonstrated a dramatic reduction of their concentrations. Therefore, pyrolysis qualified to efficiently eliminate most of the target compounds in the final char. Further research is expected to assess whether this promising performance compensates for the high energy costs associated.

Grab samples of sewage sludge collected in three different STPs have been analyzed after different steps of the treatment. Occurrence and evolution of individual compounds have been discussed, as well as their overall trends as BTRs and BTHs families. Some compounds were degraded (or transformed), other showed recalcitrant properties, and some of them even increase their concentration along the treatment. Different TPs have been proposed. However, the lack of information about transformation pathways and the absence of some BTRs and BTHs in the present study hindered the possibility of finding a clear explanation for each case. To the best of our knowledge, this is the first study reporting concentrations of 2-M-BTH and 2-Me-BTH in sewage sludge. Moreover, mass loads have been estimated, showing that high amounts of BTRs and BTHs are disposed every year into landfills or applied to agricultural soils, when the sewage sludge is used as fertilizer. In particular, an average between 58 and 2,313 Kg of BTRs, and 335 and 5,056 Kg of BTHs is released into the environment every year, because of the sludge produced in the STPs. Additionally, the coefficient of distribution (K_d) between particulate and dissolved phase has been calculated. Generally, these pollutants showed a clear tendency to be adsorbed onto the sludge, except for BTH. Again, to the best of our knowledge, this was the first time that k_d values for 1-OH-BTR, 5-Cl-BTR and 2-M-BTH were reported.

In summary, a unique methodology has been successfully validated and applied for the analysis of 11 BTHs and BTRs in environmental matrices and WEEE. The obtained results contribute to a better understanding of the behavior of these compounds during different treatments and STPs. And the reported concentrations could be used to establish future regulations that limit the presence of these pollutants in the environment, due to their high and negative impact on the human health and wildlife.

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Appendices

Appendix A. Supplementary Tables

Tables S1 – S4. Absolute recoveries (AR%), matrix effects (ME%), method recoveries (MR%) and relative method recoveries (RMR%) for 5 and 10 µg L^{-1} spikes are shown in Tables S1, S2 for WEEE. Tables S3, S4 compiles the results for sewage sludge.

Table S1. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recovery (RMR%) for a fortification level of 5 µg L^{-1} in WEEE samples.

Compound	AR% (RSD%)	ME%	MR% (RSD%)	RMR% (RSD%)
1-OH-BTR	62 (44)	-17	52 (44)	74 (36)
2-ABTH	72 (14)	-29	42 (21)	60 (11)
2-M-BTH	58 (31)	-42	26 (42)	46 (30)
2-Me-BTH	69 (20)	-18	37 (31)	67 (18)
2-MeS-BTH	41 (18)	15	17 (59)	30 (65)
5-Cl-BTR	62 (29)	-39	37 (29)	53 (19)
BTR	120 (71)	-15	84 (97)	120 (47)
BTR-COOH	90 (23)	-33	69 (23)	87 (13)
TTR	72 (40)	-27	44 (49)	63 (39)
XTR	59 (34)	-31	41 (34)	59 (24)

Table S2. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recovery (RMR%) for a fortification level of 10 µg L^{-1} in WEEE samples.

Compound	AR% (RSD%)	ME%	MR% (RSD%)	RMR% (RSD%)
1-OH-BTR	69 (4)	-5	66 (4)	111 (8)
2-ABTH	80 (6)	-23	58 (7)	96 (10)
2-M-BTH	90 (26)	-38	52 (28)	115 (13)
2-Me-BTH	74 (29)	-23	48 (34)	107 (17)
2-MeS-BTH	50 (34)	11	42 (48)	94 (46)
5-Cl-BTR	68 (29)	-37	43 (29)	71 (21)
BTR	78 (27)	-18	54 (34)	89 (27)
BTR-COOH	82 (19)	-16	69 (19)	115 (17)
TTR	72 (26)	-22	52 (28)	86 (19)
XTR	72 (8)	-40	43 (8)	72 (5)

Table S3. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recovery (RMR%) for a fortification level of 5 µg L^{-1} in sewage sludge samples.

Compound	AR% (RSD%)	ME%	MR % (RSD%)	RMR% (RSD%)
1-OH-BTR	50 (11)	14	42 (15)	54 (19)
2-ABTH	53 (25)	12	55 (27)	70 (25)
2-M-BTH	51 (16)	-44	28 (17)	52 (22)
2-Me-BTH	42 (4)	41	60 (5)	114 (3)
2-MeS-BTH	62 (14)	160	17 (140)	33 (138)
5-Cl-BTR	53 (20)	-30	35 (21)	45 (22)
BTR	103 (4)	292	31 (85)	40 (46)
BTR-COOH	73 (33)	79	85 (51)	109 (53)
TTR	90 (23)	620	-198 (-75)	-254 (-78)
XTR	88 (31)	108	17 (331)	22 (385)

Table S4. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recovery (RMR%) for a fortification level of 10 µg L^{-1} in sewage sludge samples.

Compound	AR% (RSD%)	ME%	MR% (RSD%)	RMR% (RSD%)
1-OH-BTR	70 (13)	6	67 (14)	85 (21)
2-ABTH	70 (10)	2	70 (11)	89 (17)
2-M-BTH	77 (2)	-48	40 (2)	75 (5)
2-Me-BTH	57 (9)	13	66 (9)	125 (6)
2-MeS-BTH	47 (18)	85	19 (86)	36 (82)
5-Cl-BTR	70 (11)	-37	43 (11)	56 (17)
BTR	92 (10)	136	48 (45)	62 (52)
BTR-COOH	81 (10)	70	116 (12)	149 (18)
TTR	215 (17)	137	96 (89)	123 (89)
XTR	85 (15)	22	35 (45)	44 (43)

Tables S5-S6. AR%, R%, ME%, and MR% for the compounds that did not pass the validation test are shown in Tables S5 and S6 (WEEE and sludge, respectively).

Table S5. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recoveries (RMR%) for the compounds which are not semi-quantified. Values for 5, 10 and 20 fortification levels in WEEE.

Compound	Fortification level (µg L^{-1})	AR% (RSD%)	ME%	MR% (RSD%)	RMR% (RSD%)
2-Cl-BTH	5	12 (173)	-134	-22 (-25)	-39 (-12)
	10	31 (140)	-102	-4 (-303)	-10 (-454)
	20	112 (173)	-110	-4 (-39)	-6 (-47)
2-OH-BTH	5	112 (4)	63	-524 (-27)	-9333 (-28)
	10	48 (18)	460	-220 (-30)	-494 (-38)
	20	136 (13)	-12	-16 (-189)	-26 (-176)
2-S-BTH	5	17 (53)	-166	-14 (-26)	-25 (-33)
	10	52 (144)	-121	3 (517)	3 (379)
	20	23 (53)	-116	-3 (-11)	-3 (-27)
2-SCNMeS-BTH	5	89 (47)	-246	367 (99)	653 (98)
	10	66 (24)	-432	-5 (-1498)	-12 (4627)
	20	252 (47)	-382	99 (122)	168 (112)
5-ABTR	5	0.02 (146)	-46	0.01 (146)	0.01 (144)
	10	0.01 (119)	-40	0.01 (119)	0.01 (121)
	20	0.09 (140)	-62	0.03 (140)	0.05 (139)

Table S6. Absolute recoveries (AR%), matrix effects (ME%), method recovery (MR%) and relative method recovery (RMR%) for the compounds which are not semi-quantified. Values for 5, 10 and 20 fortification level in sewage sludge.

Compound	Fortification level (µg L^{-1})	AR% (RSD%)	ME%	MR % (RSD%)	RMR % (RSD%)
2-Cl-BTH	5	160 (114)	-80	19 (192)	36 (190)
	10	37 (91)	-71	4 (267)	7 (256)
	20	6 (173)	-78	-1 (-250)	-2 (-281)
2-OH-BTH	5	92 (16)	564	-163 (-99)	-306 (-96)
	10	160 (7)	116	91 (56)	172 (54)
	20	124 (5)	88	94 (18)	176 (21)
2-S-BTH	5	23 (41)	-60	5 (88)	9 (87)
	10	13 (20)	-79	1 (80)	1 (77)
	20	12 (55)	-77	2 (81)	4 (77)
2-SCNMeS-BTH	5	31 (8)	-82	5 (9)	10 (15)
	10	12 (55)	-71	12 (9)	23 (7)
	20	38 (24)	-73	10 (24)	21 (24)
5-ABTR	5	6 (9)	-35	0.1 (589)	0.1 (515)
	10	3 (39)	-50	-0.2 (-311)	-0.4 (-327)
	20	2 (78)	-53	0.2 (339)	0.4 (374)

Compounds showed in in Table S5 and S6 did not meet the quality requirement during validation and were ruled out from the analytical method.

Linearity was investigated in the range from 0 – 20 µg L^{-1} considering an injection volume of 5 µL. Hence, corrected signal response (analyte peak area divided by IS peak area) was plotted versus spiked concentration. A linear model fitted along that range with determination coefficients above 0.9745. iLOQ levels were between 0.05 and 5 µg L^{-1}. Therefore, sensitivity of the instrument was not an issue.

2-Cl-BTH and 2-S-BTH were not detected in the spikes (pre-extraction nor post-extraction then, the method was not suitable for this compound. In case of 2-SCNMeS-BTH and 2-OH-BTH, high contamination in the blanks was found, thus, their quantification was not possible either. 5-ABTR was not present in the spiked samples (before extraction), but good response was presented in the matrix matched (spikes post-extraction), which could be related with a very poor sample pretreatment recovery. pKa for this compound is quite high (9.61), thus, it might have remained retained in the cartridge. The elution volume should be further optimized for this analyte.

Some methodological improvements could be proposed. Hence, a more exhaustive clean-up step in order to further reduce the interferences in the matrix, and thus, reduce the associated ME%. Regardless, a good compromise solution was accepted with this methodology, as it is able to quantitatively extract more than 40 compounds belonging to very diverse physico-chemical featured families.

Table S7. Determined BTRs and BTHs concentrations in particulate phase (ng g^{-1}).

		BTRs						BTHs				
	Sample	**1-OH-BTR**	**5-Cl-BTR**	**BTR**	**BTR-COOH**	**TTR**	**XTR**	**2-ABTH**	**2-M-BTH**	**2-Me-BTH**	**2-MeS-BTH**	**BTH**
STP-1	**R_(1)_P_1**	n.d.[a]	<LOQ[b]	n.d.	n.d.	n.d.	177	<LOQ	25	n.d.	168	<LOQ
	R_(1)_P_2	51	2.1	5.6	n.d.	1.8	283	1.4	5.4	n.d.	151	540
	R_(1)_P_3	238	<LOQ	4,881	n.d.	244	303	<LOQ	5.0	n.d.	152	n.d.
	P_(1)_P_1	n.d.	4.1	866	<LOQ	965	122	n.d.	8.3	66	281	907
	P_(1)_P_2	n.d.	9.2	332	n.d.	486	113	n.d.	<LOQ	100	239	1,104
	P_(1)_P_3	16	2.1	242	n.d.	345	80	n.d.	n.d.	65	204	747
STP-2	**P_(2)_P_1**	64	6.1	n.d.	n.d.	26	43	n.d.	10	56	237	n.d.
	P_(2)_P_2	41	6.1	n.d.	n.d.	32	42	n.d.	6.7	50	210	n.d.
	P_(2)_P_3	7.1	2.8	n.d.	n.d.	97	97	<LOQ	12	59	217	n.d.

[a]Non-detected. [b]Under the limit of quantification.

Appendix B. Supplementary Figures.

Figure S1. Effect of the extract acidification (Formic acid, 0.1%, v/v) prior the UHPLC-MS/MS analysis.

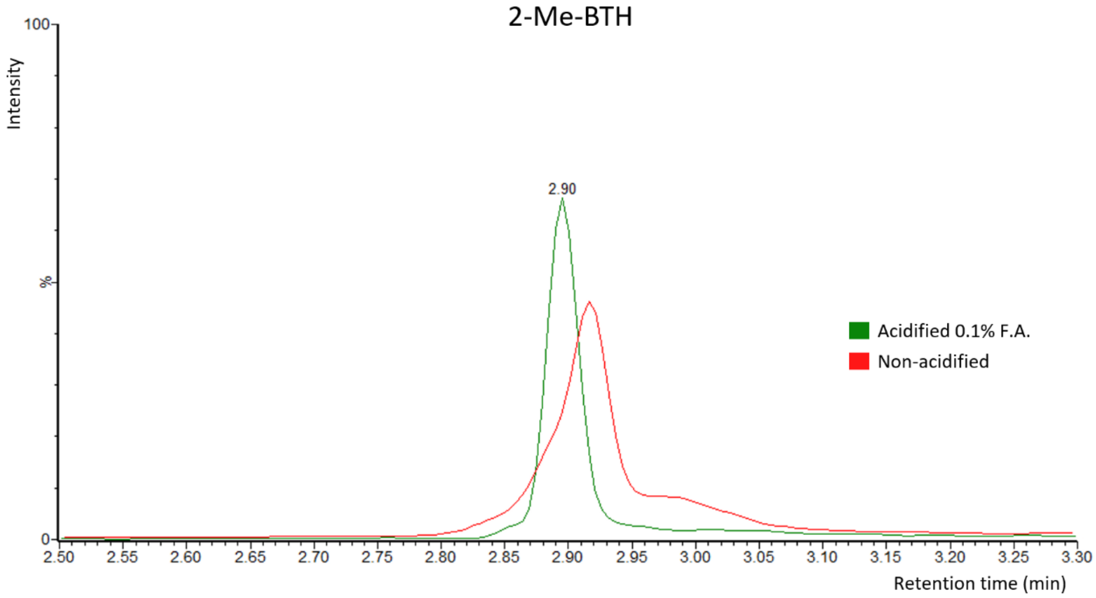

Figure S1. Differences between acidified (green) and non-acidified (red) standard of 50 µg L^{-1}.

The resulting chromatographic peak after acidification was thinner and presented less tail as showed in Figure S1 for 2-Me-BTH (fortification level: 50 µg L^{-1}) as an example. This improvement enabled a more reliable quantification and better limits of quantification.

Figures S2-S17. Internal standard calibration curves for all the studied compounds in MeOH:Deionized water (1:1, v/v) are shown in Figures S2 – S17. Additionally, coefficient of determination (R^2) and linear equation are included. Concentrations (µg L^{-1}) are represented in abscissa axis. The ratio between the analyte (A) and the internal standard (A_{IS}) areas are represented in Y-axis.

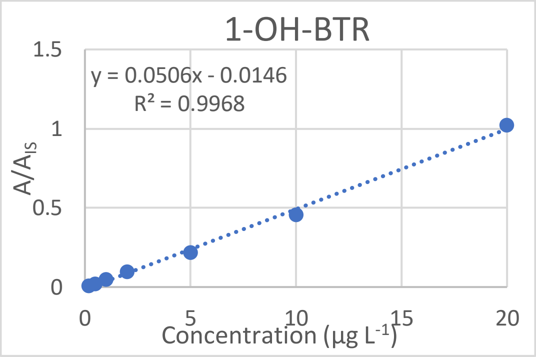

Figure S2. Calibration curve, R^2 and linear equation for 1-OH-BTR in MeOH/Deionized water 1:1 (v/v).

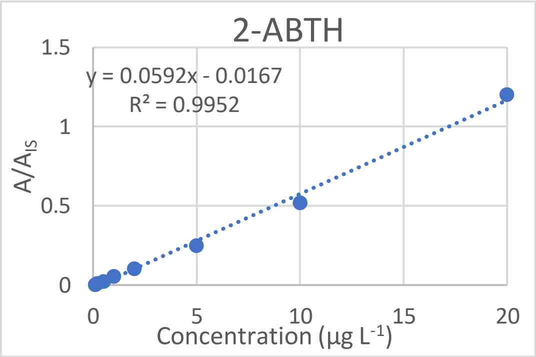

Figure S3. Calibration curve, R^2 and linear equation for 2-ABTH in MeOH/Deionized water 1:1 (v/v).

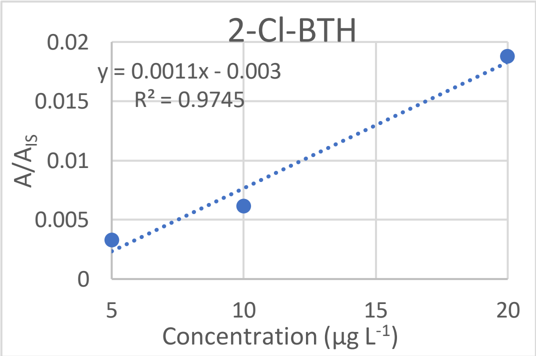

Figure S4. Calibration curve, R^2 and linear equation for 2-Cl-BTH in MeOH/Deionized water 1:1 (v/v).

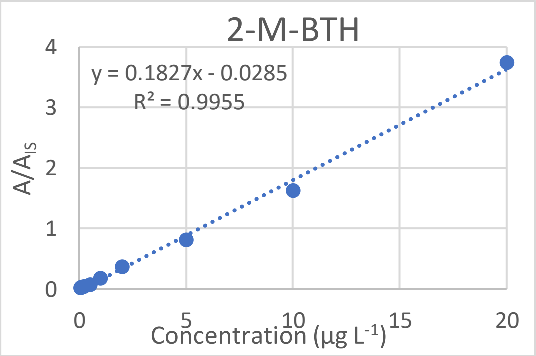

Figure S5. Calibration curve, R^2 and linear equation for 2-M-BTH in MeOH/Deionized water 1:1 (v/v).

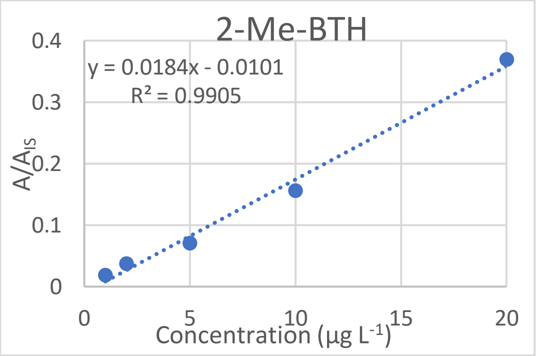

Figure S6. Calibration curve, R^2 and linear equation for 2-Me-BTH in MeOH/Deionized water 1:1 (v/v).

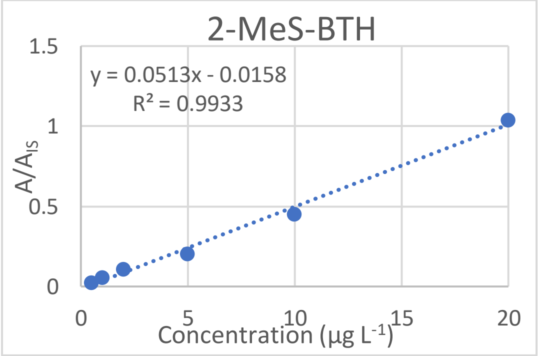

Figure S7. Calibration curve, R^2 and linear equation for 2-MeS-BTH in MeOH/Deionized water 1:1 (v/v).

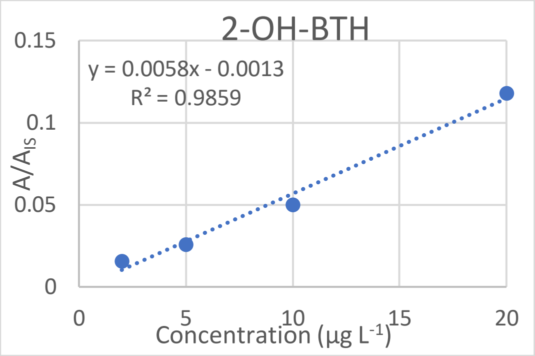

Figure S8. Calibration curve, R^2 and linear equation for 2-OH-BTH in MeOH/Deionized water 1:1 (v/v).

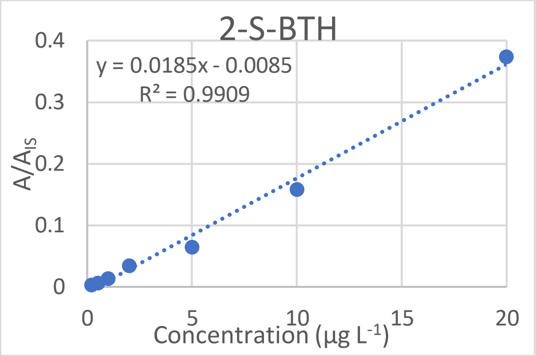

Figure S9. Calibration curve, R^2 and linear equation for 2-S-BTH in MeOH/Deionized water 1:1 (v/v).

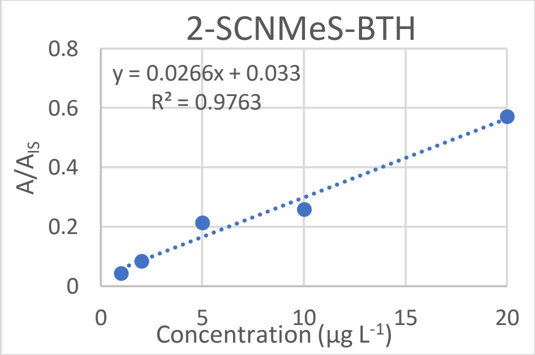

Figure S10. Calibration curve, R^2 and linear equation for 2-SCNMeS-BTH in MeOH/Deionized water 1:1 (v/v).

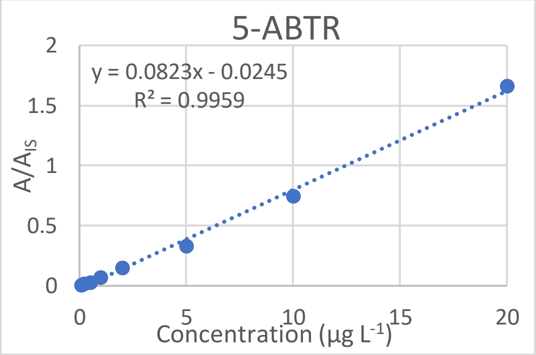

Figure S11. Calibration curve, R^2 and linear equation for 5-ABTR in MeOH/Deionized water 1:1 (v/v).

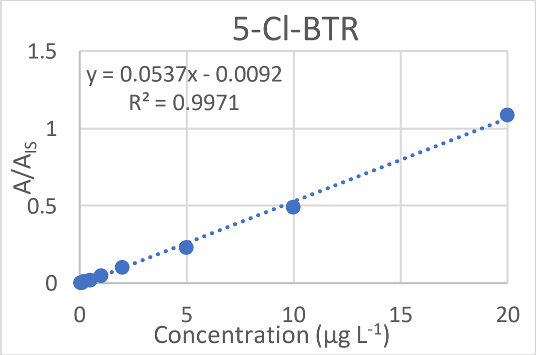

Figure S12. Calibration curve, R^2 and linear equation for 5-Cl-BTR in MeOH/Deionized water 1:1 (v/v).

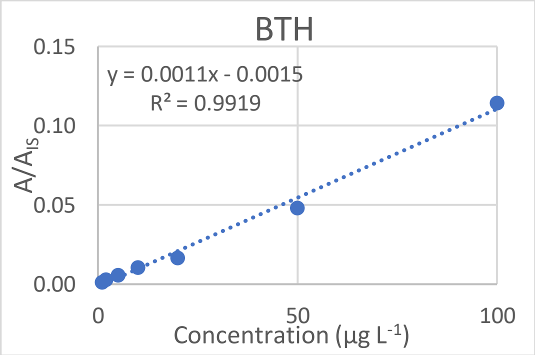

Figure S13. Calibration curve, R^2 and linear equation for BTH in MeOH/Deionized water 1:1 (v/v).

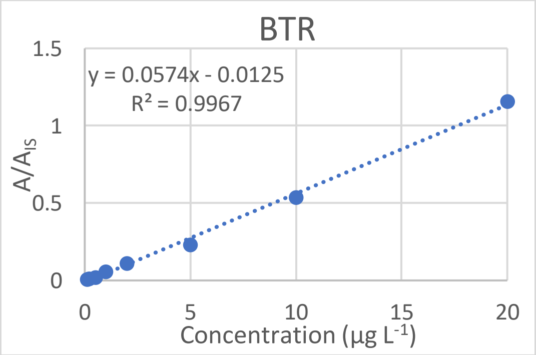

Figure S14. Calibration curve, R^2 and linear equation for BTR in MeOH/Deionized water 1:1 (v/v).

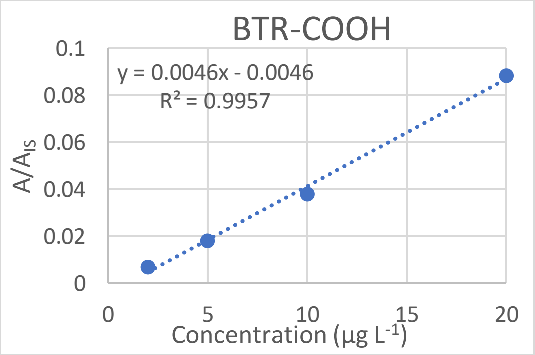

Figure S15. Calibration curve, R^2 and linear equation for BTR-COOH in MeOH/Deionized water 1:1 (v/v).

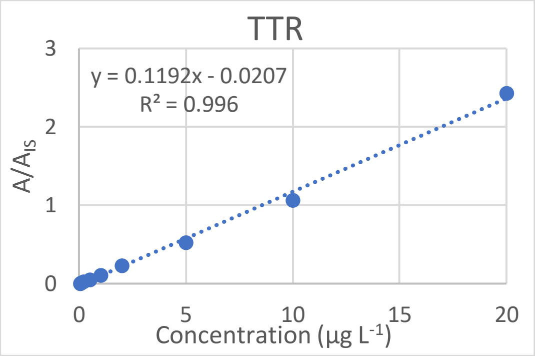

Figure S16. Calibration curve, R^2 and linear equation for TTR in MeOH/Deionized water 1:1 (v/v).

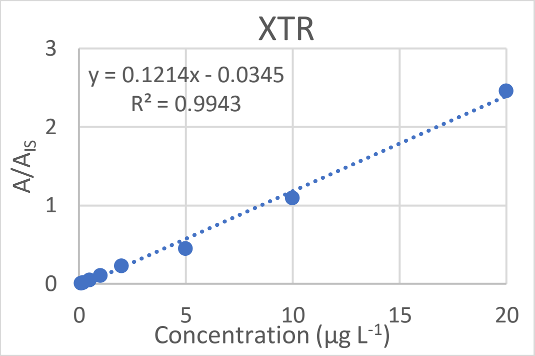

Figure S17. Calibration curve, R^2 and linear equation for XTR in MeOH/Deionized water 1:1 (v/v).

Figure S18. Comparison among external calibration versus post and pre-extraction spikes.

Matrix effect as well as the sample treatment affected the peak area produced for a same amount of analyte. This is presented in Figure S18 for TTR in WEEE matrix, where the external calibration curve showed the highest slope, followed by the post-extraction spikes and finally de pre-extraction spikes. Slope is related to sensitivity.

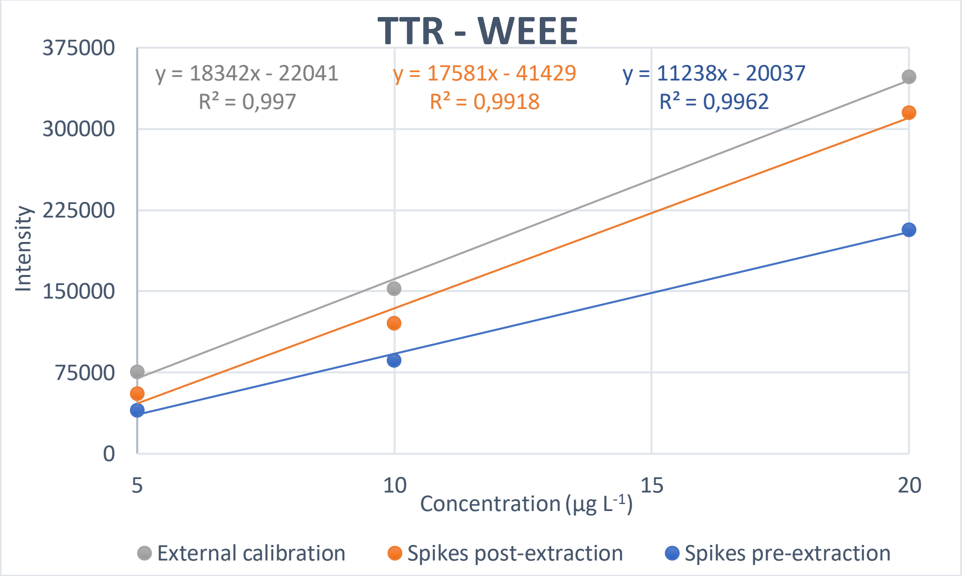

Figure S18. Comparation of the intensity for the external calibration, the spikes post-extraction and the spikes pre-extraction.

This is consistent to the fact that external calibration samples did not contain matrix or undergo any analytical process. Signal in post-extraction spikes only registered signal withdrawal associated to ESI matrix effect.

Figure S19. Peak areas for BTH (post-extraction spikes) at 3 fortification levels.

The quantification of BTH was hindered by its degradation (*Section 3.2 Method validation*). Additionally, two peaks were present in the chromatogram of both BTH transitions (Q_1 and Q_2). Identification of the correct retention time (RT) was necessary. To achieve this, a post-extraction spike was performed at 3 fortification levels (12.5, 50 and 100 µg L^{-1}) (Figure S19).

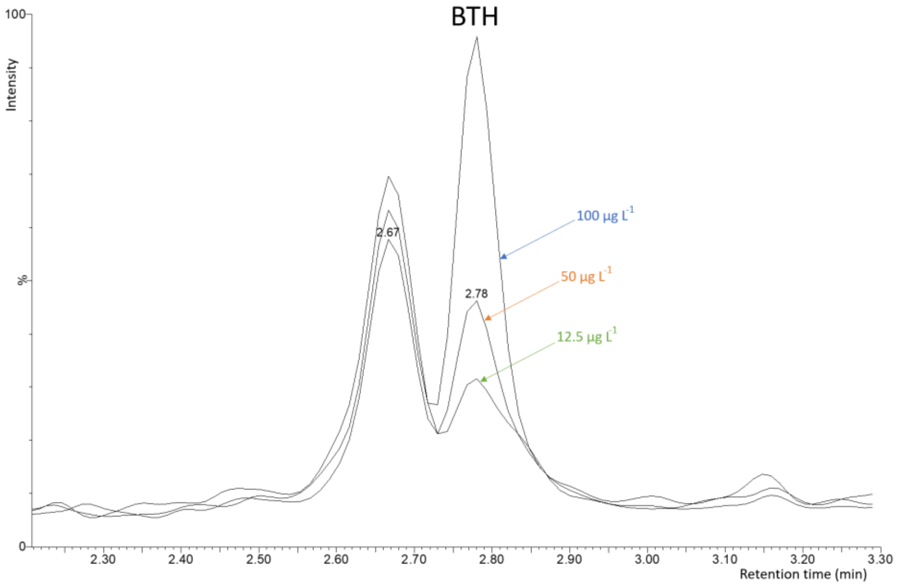

Figure S19. Peak areas for BTH (post-extraction spikes) at 3 fortification levels.

The area under the peak at 2.78 min increased with the concentration. In contrast, the peak at 2.67 min did not vary significantly. For this reason, a retention time of 2.78 min was assigned to BTH in the described technical conditions.

Figures S20 – S49. Compound occurrence evolution during the sewage sludge treatment in each STP. Concentrations are presented in the Y-axis in ng L^{-1} for STP-1 and STP-2, and in ng g^{-1} for STP-3. In the X-axis, the samples are represented.

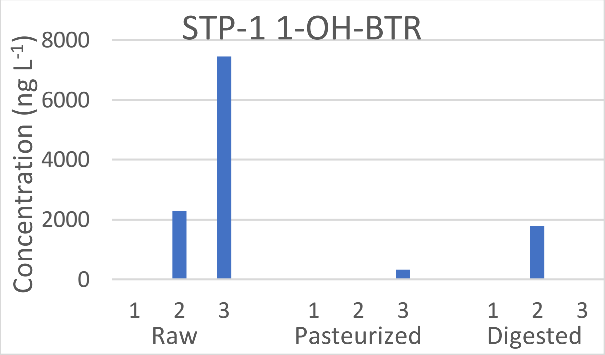

Figure S20. Evolution of 1-OH-BTR in STP-1.

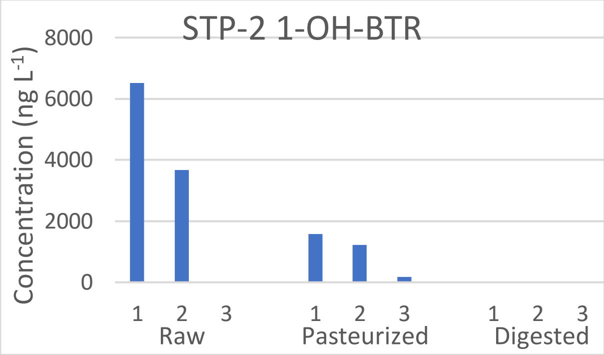

Figure S21. Evolution of 1-OH-BTR in STP-2.

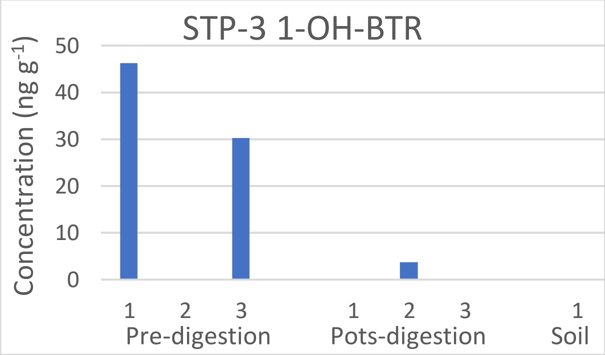

Figure S22. Evolution of 1-OH-BTR in STP-1.

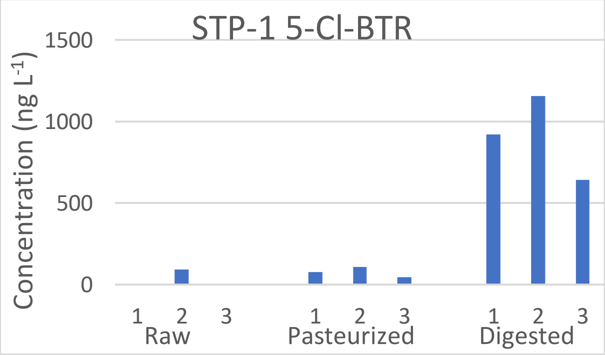

Figure S23. Evolution of 5-Cl-BTR in STP-1.

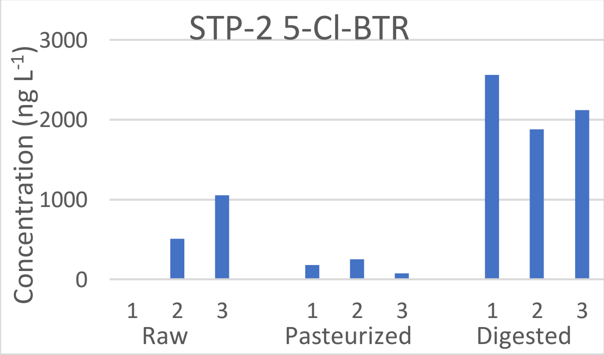

Figure S24. Evolution of 5-Cl-BTR in STP-2.

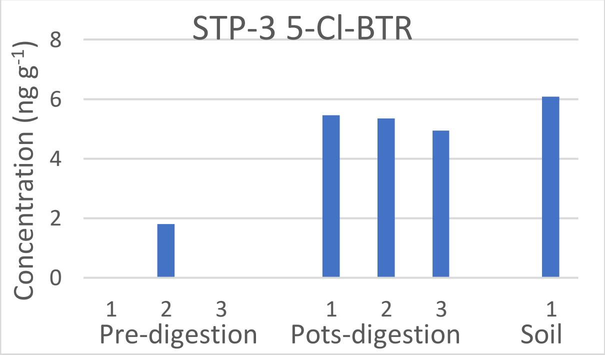

Figure S25. Evolution of 5-Cl-BTR in STP-3.

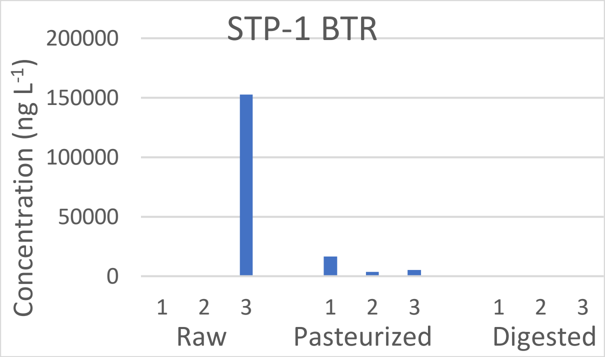

Figure S26. Evolution of BTR in STP-1.

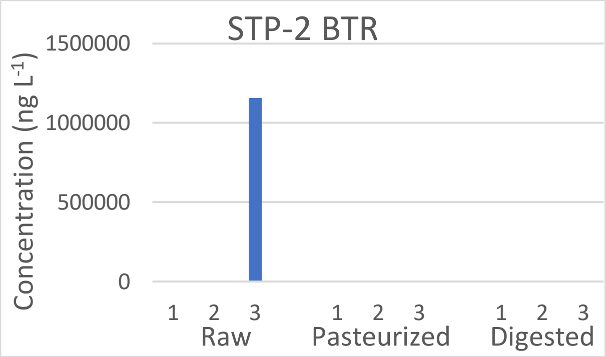

Figure S27. Evolution of BTR in STP-2.

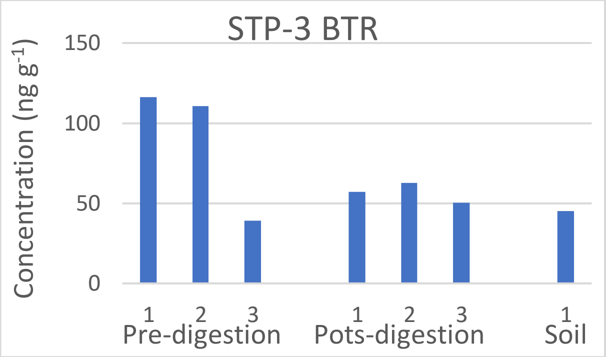

Figure S28. Evolution of BTR in STP-3.

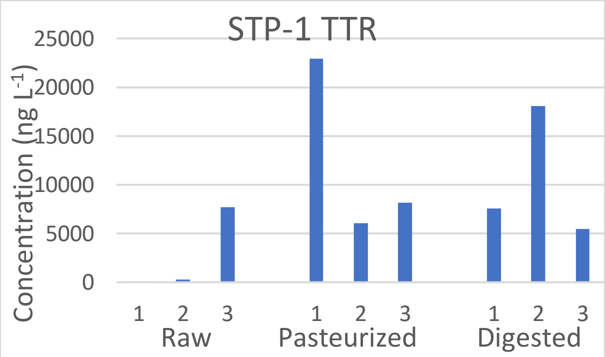

Figure S29. Evolution of TTR in STP-1.

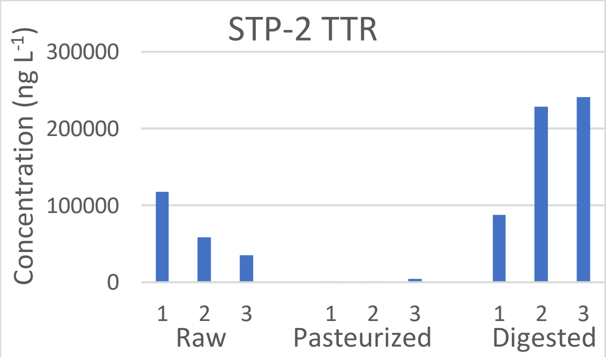

Figure S30. Evolution of TTR in STP-2.

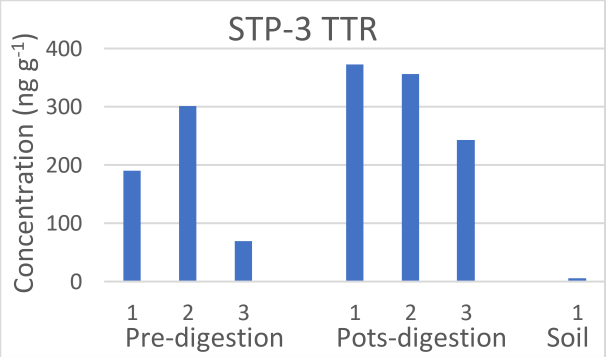

Figure S31. Evolution of TTR in STP-3.

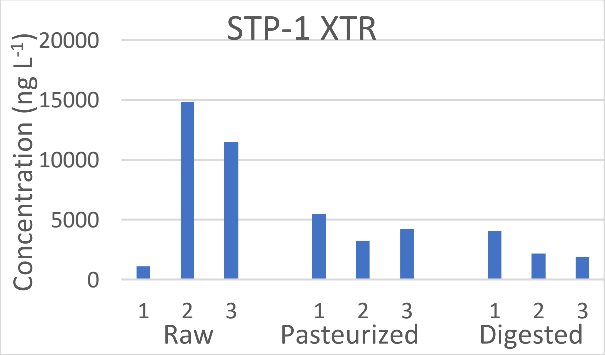

Figure S32. Evolution of XTR in STP-1.

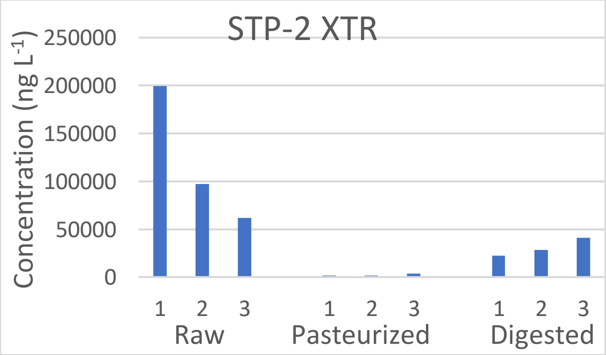

Figure S33. Evolution of XTR in STP-2.

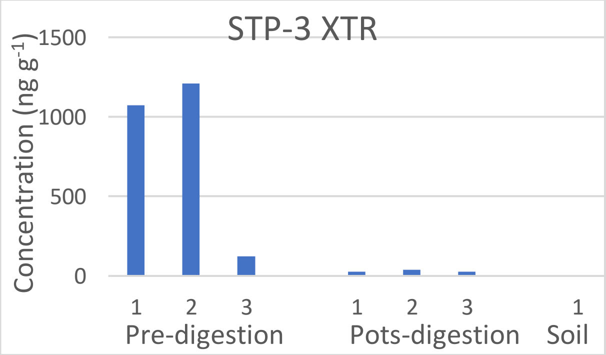

Figure S34. Evolution of XTR in STP-3.

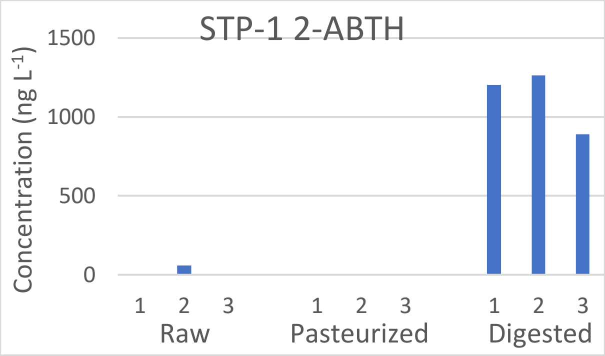

Figure S35. Evolution of 2-ABTH in STP-1.

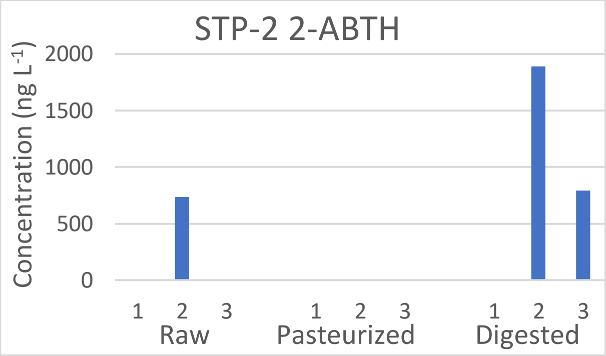

Figure S36. Evolution of 2-ABTH in STP-2.

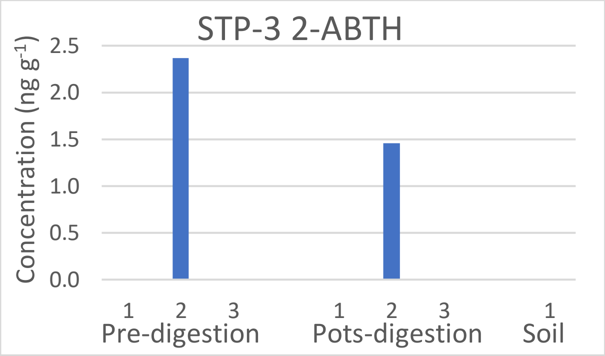

Figure S37. Evolution of 2-ABTH in STP-3.

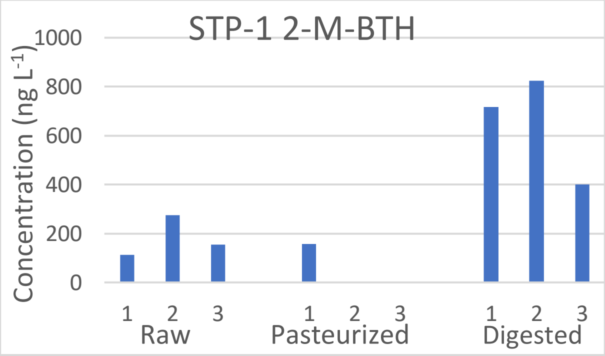

Figure S38. Evolution of 2-M-BTH in STP-1.

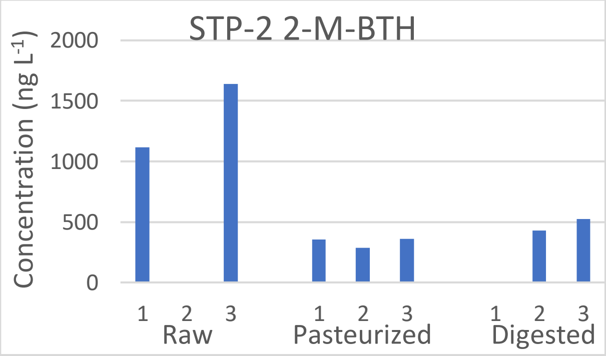

Figure S39. Evolution of 2-M-BTH in STP-2.

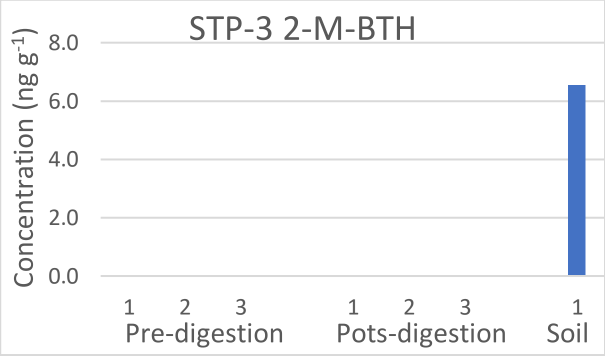

Figure S40. Evolution of 2-M-BTH in STP-3.

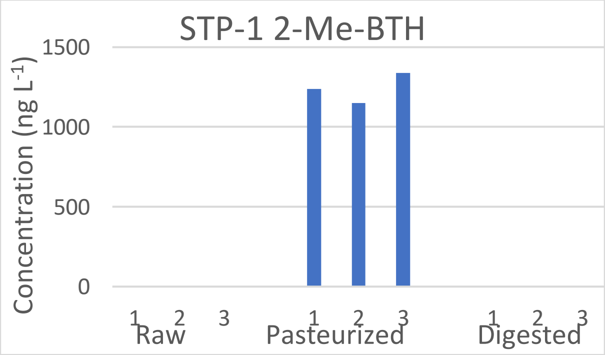

Figure S41. Evolution of 2-Me-BTH in STP-1.

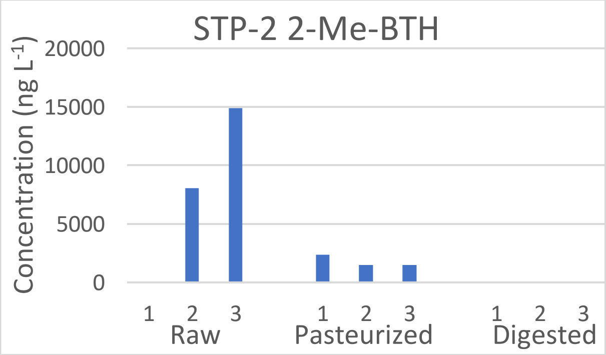

Figure S42. Evolution of 2-Me-BTH in STP-2.

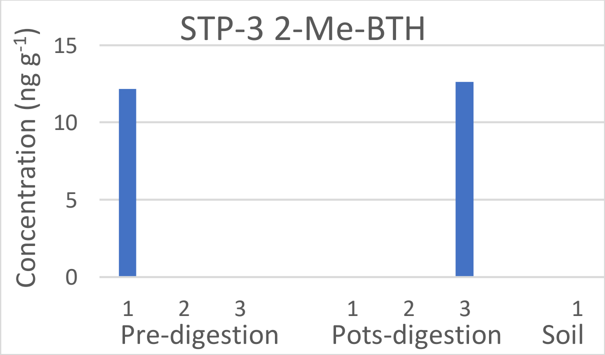

Figure S43. Evolution of 2-Me-BTH in STP-3.

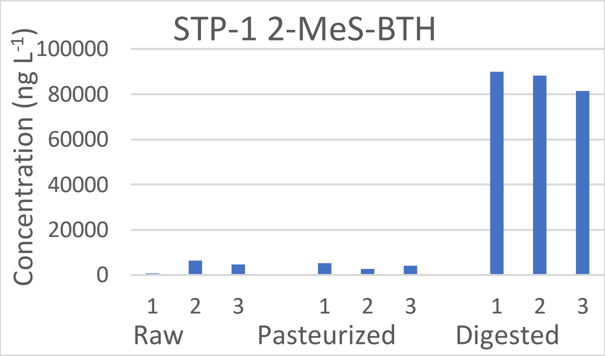

Figure S44. Evolution of 2-MeS-BTH in STP-1.

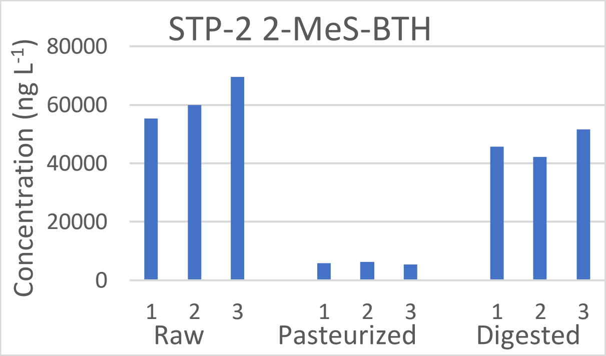

Figure S45. Evolution of 2-MeS-BTH in STP-2.

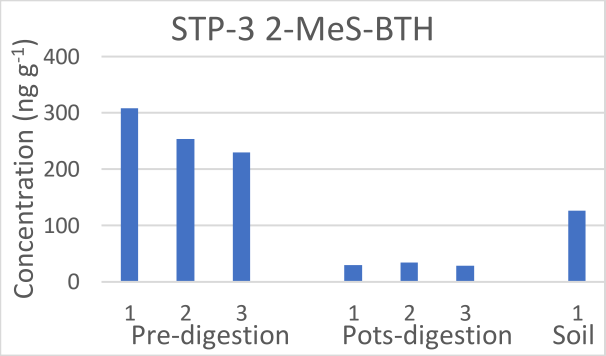

Figure S46. Evolution of 2-MeS-BTH in STP-3.

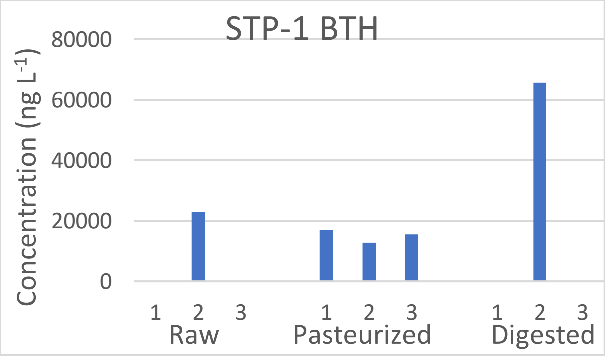

Figure S47. Evolution of BTH in STP-1.

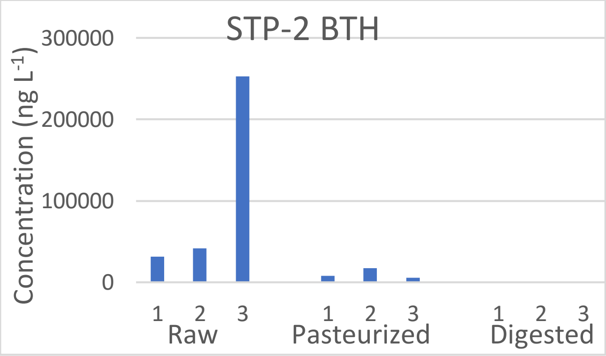

Figure S48. Evolution of BTH in STP-2.

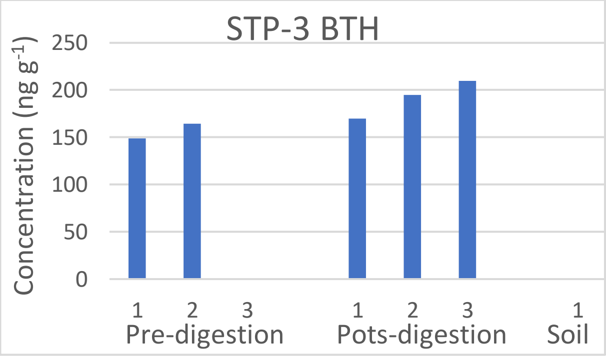

Figure S49. Evolution of BTH in STP-3.