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Fate of glyphosate and its metabolite AminoMethylPhosponic acid (AMPA) from point source through wastewater sludge and advanced treatment

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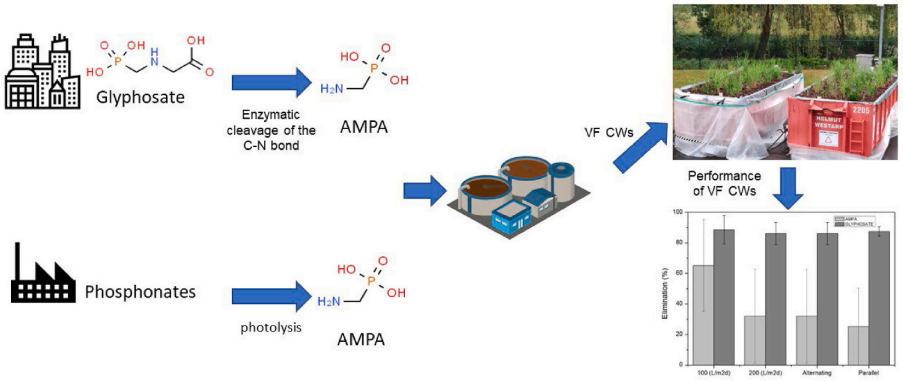
HIGHLIGHTS

- Non-agricultural application of glyphosate is relevant for urban emission.
- The origin of AMPA is mostly related to industry rather than glyphosate.
- Concentrations of AMPA and glyphosate are relevant in reject water.
- Vertical Flow Constructed Wetland is 90% efficient towards glyphosate.
- AMPA degradation in the soil is sensitive to the wetland loading regime.

GRAPHICAL ABSTRACT



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ABSTRACT

The fate of glyphosate and its metabolite AminoMethylPhosponic acid (AMPA) was followed at the catchment of the Sûre river, mainly characterized by small population density and small and medium-sized wastewater treatment plants (WWTPs). A high concentration of AMPA was found in water samples collected in inlet from different wastewater streams, the industry being the main contributor, while glyphosate resulted mainly in domestic origin. The two molecules were also monitored in the anaerobic digestion as in the supernatant produced after centrifugation (reject water). A total of 0.0713 and 2.24 g/d of glyphosate and AMPA respectively were regularly returned to the activated sludge tank (AST) indicating a 20% impact of the sludge management line on the global wastewater mass balance. Finally, the use of Constructed Wetlands (CWs) in Vertical Flow (VF) configuration was tested as a suitable technology to retain both glyphosate and AMPA (90 and up to 50% elimination respectively) and minimize their discharge into surface water.

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1. Introduction

Glyphosate is the most used Organophosphorous pesticide (OPPs) in the European Union (EU). The total sales volume was estimated at 46,527 tonnes in 2017 representing 33% of the total weedkiller sale across the EU (Antier et al., 2020), 90% of which were used to protect plants (in agriculture) and the rest for other applications in non-cultivated areas (e.g. on railways tracks, sidewalks and lawns).

Glyphosate presents two main biodegradation pathways, one leading to the formation of AminoMethylPhosponic acid (AMPA) (cleavage of C–N bond, catalysed by an oxidase), and the other one to the formation of sarcosine and glycine (cleavage of C–P bond, catalysed by C–P lyase) (Wang et al., 2016; Zhan et al., 2018; Aslam et al., 2023), contaminating the aquatic environment via diffuse (surface runoff, erosion, spray-drift, and bioleaching) and point sources (effluent of wastewater treatment plants (WWTP), discharge from Combined Sewer Overflows (CSOs)) (Grandcoin et al., 2017).

Controversies have arisen with respect to the direct and indirect impact of glyphosate on the environment and human health. As for other OPPs, it has been demonstrated that glyphosate is able to bioaccumulate, and it is considered potentially toxic to a wide variety of species (Geetha, 2021) including humans (Portier et al., 2016). Same accounts for the metabolite AMPA, which has been indicated as genotoxic (Mañas et al., 2009).

However, studies appraising the impact of the active ingredient and its commercial formulations have been challenging due to the contradictory scientific evidence. As for now, the EU extended the five years approval for glyphosate's use until December 15, 2023 (EC, 2022) when the peer review process will be completed.

Glyphosate and AMPA were often detected in WWTP's effluents (Poiger et al., 2020). Rainfall events are known to increase the transport of glyphosate by runoff on concrete and asphalt surfaces (Gandhi et al., 2021), which facilitates its transfer to sewers. The impact of this contamination is especially relevant in case of WWTPs with combined sewer systems which confirms WWTP effluents being a major urban source of glyphosate pollution towards surface water (Botta et al., 2009). Glyphosate is known to quickly degrade in soil under oxic conditions and the microflora is then responsible for the formation of AMPA (Grandcoin et al., 2017). However, AMPA is also a photodegradation product of amino-polyphosphonates, which are used in both industry and household for a wide range of applications as detergents, flame retardants, complexing agents in textile and paper industries. Thus, its presence in surface waters cannot be related to the use of glyphosate only.

While the fate of glyphosate in agricultural soils has been widely investigated (Vymazal and Brezinová, 2015; Suciu et al., 2023), origin and transformation pathways of both glyphosate and AMPA in an urban context is still not well described although it is essential for future effective pesticide management and low phosphonate concentrations discharge, known for contributing to eutrophication due to the abiotic degradation (Rott et al., 2020).

Most of the research on the degradation of glyphosate by microorganisms rarely focused on the application of activated sludges as mixed cultures especially not at full scale (Feng et al., 2020). Even less about their behaviour in the anaerobic digestion of sewage sludge together with their presence in the returned supernatant (reject water) has been reported so far because the effect of pollution was rather studied for the disposed sludge used in agriculture. Because of their high concentrations discharged from WWTPs, several processes have been considered for their removal. Among these technologies, Constructed Wetlands (CWs) have been previously demonstrated to be a low cost alternative for non-point-source pollution of glyphosate, in particular agricultural run off (Imfeld et al., 2013; Liu et al., 2019; Braschi et al., 2022) but their application as post-treatment step towards OPPs is inadequately described. In the context of the Interreg Greater Region project EmiSûre, CWs are investigated for the mitigation of micropollutants (with OPPs among them) in rural areas similar to the Sûre catchment, a river acting as political boundary between Luxembourg and Germany.

This study aimed at improving the understanding of glyphosate and AMPA fates from point source to safe discharge. The main objectives are thus: (1) to investigate the possible origin of AMPA with respect to the presence (or not) of glyphosate in domestic wastewater (2) to understand both AMPA and glyphosate elimination in conventional activated sludge, physical separation, anaerobic digestion particularly quantifying the supernatant loads and the implications in the mass balance (3) finally to understand the OPPs behaviour in CWs in Vertical subsurface Flow (VF) configuration when applied as treatment solution for small and medium sized wastewater treatment effluents.

2. Materials and methods

2.1. Target compounds

Herbicide glyphosate (CAS 1071-83-6) and its main metabolite AminoMethylPhosphonic acid (AMPA) (CAS 1066-51-9) are the focus of this study. Three known compounds have been additionally selected to better understand their fate and thus used as a control: the anti-inflammatory diclofenac with CAS 15307-86-5, the anaesthetic lidocaine with CAS 137-58-6 and the flame retardant Tris(2-chloroisopropyl)phosphate (TCPP) with CAS 115-96-8. Volatility, octanol/water partitioning coefficient (K_{OW}), organic carbon partitioning coefficient (K_{OC}), acid dissociation constant (pK_a) and indications about their environmental persistency and toxicity are reported in Table S1 of the Supplementary Information.

2.2. Experimental unit setup

2.2.1. The WWTP of Echternach

The WWTP of Echternach is situated in the eastern part of Luxembourg, discharging treated effluent into the Sûre river, the physical border with Germany. It has a nominal design capacity of 36000 PE, 12500 PE of which as German wastewater stream.

The conventional activated sludge system consists of a primary sedimentation tank (V = ca. 380 m^3) and two AST (V = ca. 4500 m^3 each) integrated with a clarifier (V = ca. 3800 m^3), for a total Sludge Retention Time (SRT) of 19 d and a maximum Hydraulic Retention Time (HRT) of 31 h. The plant is equipped with grid, sand and grease trap preceding the primary sedimentation tank. The sludge treatment line is supported by an anaerobic digester (DIG) which allows to generate energy from the produced biogas (500 m^3 per day). The biogas produced is either used for the heating (WWTP buildings and the digesters) or used for electrical purposes after the cogeneration.

2.2.2. Description of the vertical flow constructed wetlands

Two pilot scale constructed wetlands of subsurface vertical flow configuration VF1 and VF2 (11,18 m^2 and 12,78 m^2 surface area, respectively), were filled with a mix of bentonite sand (grain size 0–3 mm, Liapor, Germany) and activated biochar 15% (grain size 2–5 mm, Palaterra, Germany), previously demonstrated to be suitable as post treatment step for the removal of micropollutants (Venditti et al., 2022).

The wetlands were planted with *Phragmites australis* (Verlicchi et al., 2013; Zhang et al., 2017), *Lythrum salicaria* (Brunhoferova et al., 2021) and *Iris pseudacorus* (Huang et al., 2018) at a density of 25 plants steams per m^2. The effluent of the WWTP was pumped to the wetlands as influent and equally distributed along the surface. At each loading in intermittent regime (six short daily water cycles occurring every 4 h), the wastewater flooded the wetland surface and was collected in a 50 L plastic tank after percolating through the wetland body (percolation time: up to 24 h, see S.2 of the Supplementary Information).

The feeding strategy was designed to explore two possible scenarios (Fig. 1).

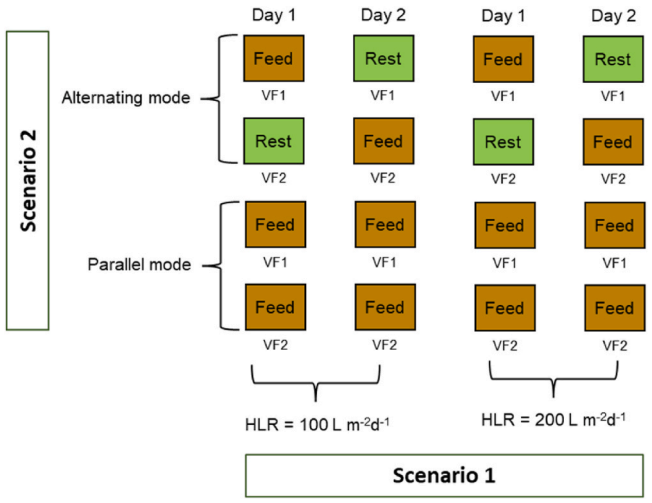

Fig. 1. Operational scheme for the pilot plant.

2.2.2.1. Scenario 1: influence of the loading. The applied Hydraulic Loading Rate (HLR) varies from 100 to 200 L m^{-2} d^{-1} and corresponds to an Organic Loading Rate (OLR) between 1.6 and 63 g Chemical Oxygen Demand (COD) d^{-1} m^{-2}.

2.2.2.2. Scenario 2: efficiency of parallel towards alternating operation. The wetlands can be operated in parallel - meaning both fed simultaneously - or in alternating mode - so that one wetland is fed while the other is rested. Alternating feeding is supposed to help in controlling the growth of the biomass attached to the medium (especially important when the wetland is used as sole treatment of domestic wastewater) and to enhance aerobic conditions for the reduction of persistent compounds.

2.2.3. Sampling design

The wetlands were monitored over 14 months of operation (see Fig. 2 for sampling locations) in order to.

- evaluate the contribution of the industrial (I) and the cross-border streams (both Luxembourgish, L and German, G) into the influent. To this end, individual raw wastewater samples were collected from each stream. Mass loads (ML) in g d^{-1} and normalized per capita (ML_N) (g d^{-1} PE^{-1}) of glyphosate and AMPA were calculated according to the following equations:

$$ML = C \cdot Q \quad (1)$$

$$ML_N = \frac{C \cdot Q}{PE} \quad (2)$$

where C is the measured pollutant concentration in ng L^{-1}, Q is the daily flow in L d^{-1}. Per capita specific load is assumed of 60 g Biological Oxygen Demand −5 days (BOD_5), 120 g Chemical Oxygen Demand (COD), and 11 g Total Kjeldahl Nitrogen (TKN) per day.

- determine the occurrence of AMPA and glyphosate: the raw wastewater (IN) was sampled after the mixing of the three streams and before entering the primary sedimentation tank.
- assess the removal rates for the conventional activated sludge treatment: effluent samples (EF) were collected after the clarifier and related to the influent for the determination of their elimination in aerobic sludge (E-AST). Elimination values (in %) are calculated as following:

$$E = \frac{Co - C}{Co} \cdot 100 \quad (3)$$

where C is the effluent and C0 is the influent concentration.

understand the fate of AMPA and glyphosate in the reject water regularly (but not continuously) recirculated: supernatant from belt filter (SBF) together with the one of the supernatant storage tank (SST) are contributing to the returned sludge (RS). The supernatant stored (SST) is composed from those of post-thickener and centrifuge (SPT and SC respectively). The removal in anaerobic digestion (E-DIG) is quantified by comparing the digester inlet and outlet, SBF and SPT respectively. The following equation was used to determine the mass balance of AMPA and glyphosate during wastewater and sludge treatment:

$$\mathrm{Load_{IN}} + (\mathrm{Load_{SST}} + \mathrm{Load_{RS}}) - \mathrm{Load_{EF}} = \mathrm{Load\ (Accumulated + biodegraded)} \quad (4)$$

Where $Load_X$ is generally equal to $Q_x \cdot C_x$ with Q as flow rate of the corresponding stream (L d^{-1}), C concentration of the contaminant in aqueous solution (ug L^{-1}) of a specific stream and x as IN, SST, SBF, RS and EF respectively.

- to study the behaviour of selected OPPs in VF wetland as post-treatment step: samples (VF1 and VF2) were collected after the wetlands and related to the effluent of the wastewater treatment plant.

Samples were mostly taken as composite in time-proportional regime.

For regular campaigns, 24 h composite samples were usually planned. To complete the assessment and in line with the recommendation from KomS (*KomS - Kompetenzzentrum Spurenstoffe*, n.d.) two prolonged campaigns were additionally carried out preparing 72 h composite

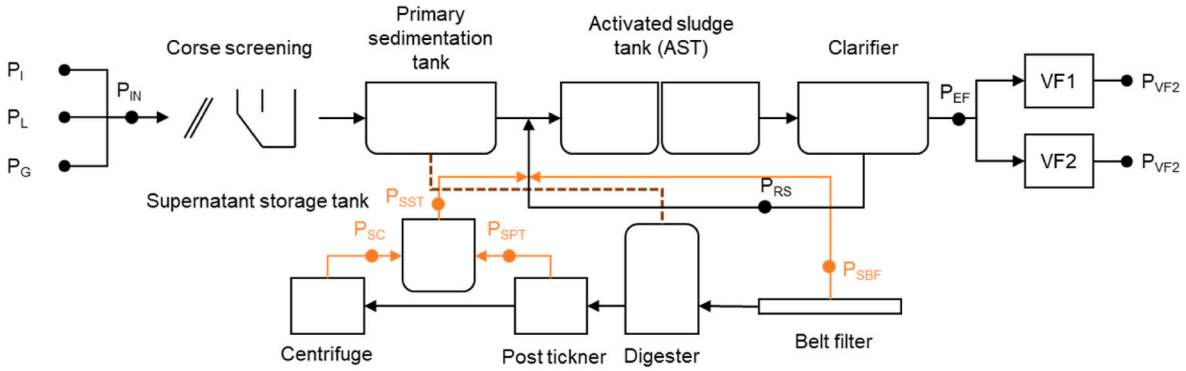

Fig. 2. Sampling points in the flow-through system: industrial, municipal Luxembourguish and municipal German streams (P_I, P_L and P_G); WWTP influent and effluent (P_{IN} and P_{EF}); recirculated sludge (P_{RS}); supernatants from belt filter (P_{SBF}), post thickener (P_{SPT}), centrifuge (P_{SC}) and supernatant storage tank (P_{SST}).

samples for IN and EF. In both regular and prolonged campaigns, an offset of 24 h was planned in collecting EF samples considering the WWTP's HRT.

Only supernatant samples (RS, SBF, SBT, SC and SST) and effluents of the wetlands (VF1 and VF2) were taken as grab samples but from larger collected volumes.

2.2.4. OPPs analysis and water chemistry

Macropollutants. COD, TN, PO_4–P, NH_4–N and NO_3–N were monitored and measure with Hach Lange cuvette tests. Oxidation-reduction potential, pH and conductivity were collected with conventional WTW (Xylem, UK) probes.

Micropollutants. The analyses of AMPA, glyphosate and control compounds were performed externally (Luxembourg Institute of Science and Technology LIST, Luxembourg) using chemical derivatization and online SPE-LC-LS/MS, with an Agilent 1200 SL coupled with a Sciex Qtrap 4500 triple quadrupole with electrospray ionization (ESI) in positive mode and negative modes. Quality parameters are reported in Table S2 of the Supplementary Information.

3. Results and discussion

3.1. Occurrence of AMPA and glyphosate in raw wastewater

High concentrations of BOD_5 and COD (Table 1) were observed in the industrial stream confirming its anthropogenic nature due to the presence of the local industry (automotive and composite materials factories etc). Significant concentrations of Na, K, Ca and conductivity as indicators of high dissolved solids or minerals were determined for the same samples. The industrial (10571 PE) stream presented half and one fourth daily flow than the Luxembourgish (2869 PE) and German (12971 PE) streams respectively.

During the sampling period, AMPA and glyphosate were both always quantified (limit of quantification in wastewater, 20 and 5 ng L^{-1} respectively). Their concentration ranges in the WWTP's influent sample (IN) collected after the screen revealed higher values for AMPA as also higher variation during the sampling period. Even if AMPA is primarily known to be the main transformation product of glyphosate in soil, it is also a metabolite of phosphonate compounds used for other anthropogenic activities. Its high concentrations measured in the industrial stream are aligned with previous studies (Grandcoin et al., 2017) which indicated phosphonate chelating agents used in industry as an alternative source of AMPA. Thus, it can be concluded that the transport of AMPA cannot be univocally described in relation to glyphosate. To better understand the significance of that, calculated mass loads were compared. When looking at the data (Fig. 3, a), the industrial stream resulted to be the main source of AMPA as in the same order of the mixed influent sample.

When the domestic load assumed as main source of glyphosate is normalized to the connected PE (Fig. 3,b), the Luxembourgish stream revealed a slightly higher contribution to both AMPA and glyphosate (0.22 and 0.082 mg PE d^{-1}) if compared to the German one (0.13 and 0.073 mg PE d^{-1}). This result implies not a significant difference in the use of glyphosate for the two countries at catchment level.

Table 1
General parameters and OPPs mean concentrations (±Standard deviation) observed in 24 h composite samples for WWTP influent (N = 12) and 72 h composite samples for the streams (N = 2).

Parameter	IN	I	L	G
Q (m^3 d^{-1})	4698 ± 1305	628 ± 105	1510 ± 105	2560 ± 1006
COD (mg L^{-1})	454 ± 165	1780 ± 206	200 ± 13	545 ± 35
BOD (mg L^{-1})	253 ± 56	1010 ± 20	114 ± 15	304 ± 17
TN (mg L^{-1})	31 ± 13	26 ± 1	27 ± 1.8	44 ± 0.7
NH4–N (mg L^{-1})	17 ± 1.8	8.5 ± 0.9	17 ± 1	28 ± 1.5
PO4–P (mg L^{-1})	1.9 ± 0.8	0.66 ± 0.1	1.3 ± 0.2	3 ± 0.9
Ptot (mg L^{-1})	4.9 ± 1.8	9.3 ± 0.8	3.2 ± 0.8	4.2 ± 1
Na (mg L^{-1})	75 ± 17	279 ± 101	60 ± 17	53 ± 19
K (mg L^{-1})	20 ± 0.8	76 ± 11	12 ± 0.8	16 ± 1
Ca (mg L^{-1})	86 ± 14	53 ± 19	100 ± 21	82 ± 13
Conductivity (uS cm^{-1})	1057	1699	1068	1008
TSS (mg L^{-1})	243 ± 145	305 ± 102	98 ± 19	108 ± 53
AMPA (ng L^{-1})	2341 ± 1658	17400 ± 1508	420 ± 37	688 ± 60
Glyphosate (ng L^{-1})	100 ± 64	73.40 ± 3	155 ± 7	370 ± 17

3.2. Fate of AMPA and glyphosate in the conventional activated sludge treatment

The concentration of AMPA and glyphosate (Table 2) were measured along the conventional treatment process to evaluate the fate pathways by activated sludge (E-AST), physical separation (dewatering by belt filter and centrifugation) and in anaerobic digestion (E-DIG). Effluent concentrations of glyphosate maintained higher than the limit of quantification confirming municipal WWTPs being an important source of discharge if compared to agricultural runoff during rain events.

3.2.1. Fate pathways in activated sludge process

Elimination of micropollutants during activated sludge process (E-AST) was calculated from influent and effluent average concentrations (Table 2). For the control compounds, the effluent concentrations were slightly higher than the influent ones. As diclofenac and lidocaine are known to be persistent and hardly removed (Falås et al., 2016; Gallé et al., 2019) having generally K_{bio} <0.1, low $LogK_{ow}$ and moderate K_d (Table S2, Supplementary Information) in conventional activated sludge treatments, a zero removal (E-AST) is assumed. For TCPP, the removal in sewage treatment plants is known to be extremely low and with tendency to be accumulated (Liang et al., 2018). The negative removal rates of AMPA have to be related to one of the two pathways proposed for glyphosate degradation, specifically the conversion of glyphosate to AMPA through the C–N cleavage. The increased AMPA concentration in the effluent are in line with literature (Feng et al., 2020): estimated that AMPA is produced with a stoichiometric ration 1:1 compared to glyphosate consumption when a kinetic test is performed using acclimated sludge.

3.2.2. Fate pathways by physical separation

Together with influent and effluent WWTP's composite samples, grab samples were collected from the several streams of supernatant contributing to the sludge returned into the activated sludge tank. The supernatants of centrifuge (SC) and post thickener (SPT) are stored in the supernatant storage tank (SST). The supernatant water (also known as reject water, or sludge liquor, or centrate or fugate) quality is highly variable depending on the dewatering unit and the specificities of the anaerobic digestion (such as T, HRT). It is typically rich in ammonium nitrogen, phosphate and exhibits low in chemical oxygen demand to nitrogen ration (COD:N < 1) (Gustavsson, 2010). Macropollutant values presented in Table 2 are thus coherent with what is expected and showed relatively higher values of COD, TN, NH_4–N and PO_4–P for SPT, directly collected after post-thickener treating the anaerobic digester effluent and suggesting the further impact by the process configuration and operational parameters of AST. Both SPT and SST seemed to equally contribute to the content of micropollutants stored in the supernatant storage tank which is then dosed in the returned sludge in a non-continuous way. This indicates that the physical separation performed from both belt filter and centrifugation does not influence the presence of the target compounds in the liquid phase and thus their partitioning as reported in very few studies (Patureau et al., 2021). The addition of cationic polymers during centrifugation seems not to induce any interaction between micropollutants and organic matter, that could affect the analytical methods while handling the samples. However, when looking at the absolute concentrations of the supernatant streams

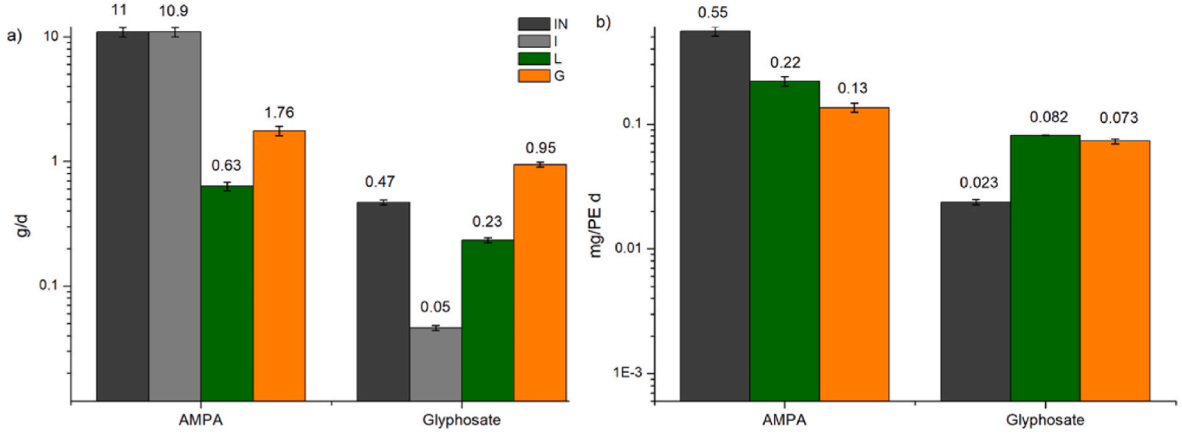

Fig. 3. Comparison of load measurements for AMPA and glyphosate in: a) the three streams (I = industrial, L = municipal Luxembourgish, G = municipal German); b) in the domestic streams only, with respect to the WWTP influent (IN).

Table 2
Mean concentrations measured (±Standard deviation) for WWTP influent and effluent (N = 12), supernatants (N = 3).

Parameter	IN	EF	RS	E-AST (%)	SC	SST	SPT	SBF	E-DIG (%)
Micropollutants (ng L^{-1})									
AMPA	2341 ± 1658	4079 ± 353	6835 ± 3231	−74	79650 ± 6576	97700 ± 8470	88300 ± 7655	5225 ± 2199	−1590
Glyphosate	100 ± 64	127 ± 5.8	303 ± 118	−27	1945 ± 657	3100 ± 142.6	3280 ± 150.9	138 ± 65.3	−2280
Diclofenac	2109 ± 115	1523 ± 80	1458 ± 763	0	1999 ± 748	1981 ± 142.6	1933 ± 101.5	1395 ± 701	−39
Lidocaine	92 ± 3.5	122 ± 4.6	184 ± 98	0	175 ± 79.4	123 ± 4.7	128 ± 4.9	112 ± 32.3	−15
TCPP	3151 ± 393.9	3002 ± 375	2911 ± 1230	0	2464 ± 398	2006 ± 250.8	2774 ± 346.8	2388 ± 628	−16
Macropollutants (mg L^{-1})									
COD	429 ± 165	15.1 ± 1	30.5 ± 0.8	96	357 ± 98	533 ± 101	1250 ± 206	92.4 ± 95	n.d.
sCOD	221 ± 15	15 ± 1	3.05 ± 0.9	93	228 ± 72	407 ± 108	1046 ± 87	68.6 ± 19	n.d.
TN	32.1 ± 1	1.03 ± 0.9	1.45 ± 0.3	97	819 ± 97	557 ± 98	639 ± 106	5.25 ± 2	−12071
NO3–N	0	0.341 ± 0.7	0.225 ± 0.1	NN	0.741 ± 0.02	1.04 ± 0.8	1.35 ± 0.3	0	n.d.
NH4–N	19.6 ± 0.8	0.179 ± 0.2	0.057 ± 0.02	99	379 ± 76	493 ± 98	447 ± 76	0.058 ± 0.02	n.d.
PO4–P	7.46 ± 0.8	1.4 ± 0.6	2.48 ± 0.4	81	86.1 ± 98	45.4 ± 9	59 ± 11	2.45 ± 0.7	−2308
Physical and chemical parameters									
pH	7.62	8.14	7.1	n.d.	7.75	8.12	7.82	7.26	n.d.
T (°C)	19.2	19.4	19.4	n.d.	25.6	14.1	14.8	19.9	n.d.
Redox	−311	107	−4	n.d.	65	85	41	52	n.d.
DO (mg L^{-1})	0.54	6.5	2.8	n.d.	4.12	4.76	2.35	4.2	n.d.
Conductivity (μS cm^{-1})	1382	1216	1209	n.d.	5.56[a]	5.31[a]	5.3[a]	1219	n.d.

[a] mS cm^{-1}, n.d. not determined.

obtained from physical separation (SC, SPT and SST), AMPA and glyphosate demonstrate to be up to 20 times higher if compared to the effluent of the WWTP as to the return sludge (Table 2). In contrary, the control compounds diclofenac, lidocaine and TCPP did not show significative variation suggesting that a change in the fate of AMPA and glyphosate occurs during the step of anaerobic digestion (DIG).

3.2.3. *Fate pathways by anaerobic digestion*

Considering an equal influent and effluent flow of the anaerobic digester, concentrations of target (AMPA and glyphosate) and control (diclofenac, lidocaine and TCPP) compounds were directly used to calculate their elimination within the process (E-DIG).

When looking at the control compounds (Table 2), the difference between outlet (SPT) and inlet (SBF) concentrations was below 30% suggesting that those compounds are unwell degraded under anaerobic conditions. This considers both measurement and propagation errors especially for lidocaine because of the low absolute concentrations.

The results of lidocaine are not in line with the few studies available in literature where the compound is expected to be highly removed because of the hydrolysis of amide (Patureau et al., 2021).

The negative mass balance of the target compounds reflects the possible production or release of molecules (biotransformation) due to biological transformation under anaerobic conditions. Biotransformation has been historically considered the main removal pathway in anaerobic digestion as the compounds generally do not accumulate on the sludge of an anaerobic bioreactor. However, biotransformation is normally due to co-metabolism: bacteria are using other contaminants at higher concentrations as their main substrates, and they activate enzymes to secondary bio-transform micropollutants (Azizan et al., 2021).

Previous studies demonstrated a linear relation between differential Total Phosphorous (TP) and glyphosate concentration (K. Zhang et al., 2015); thus, TP could be used as a suitable surrogate for the monitoring of glyphosate. The mass balance of TP in the anaerobic digestion seems to be in line with the enrichment of glyphosate and AMPA in the outlet of the digester, which both contain phosphate. As phosphorous is known to be released from activated sludge under anaerobic conditions, a similar mobilization can be assumed for the target compounds (Dai et al., 2021).

3.2.4. *Process mass balance of AMPA and glyphosate*

The results of Table 2 allowed to present a mass balance which is based on the assumption that the concentration of both AMPA and glyphosate can be neglected in the particulate phase because Log K_{ow} < 2.5 and consequently Kd·TSS <0.1. The tool SimpleTreat (Rivm, NL) has been used to model the mass balance of AMPA and glyphosate as if both surplus and settle sludge would not be directed to the anaerobic digester (Fig. 4, a). The model estimates the removal of both AMPA and glyphosate considering their physical and chemical properties as input. The

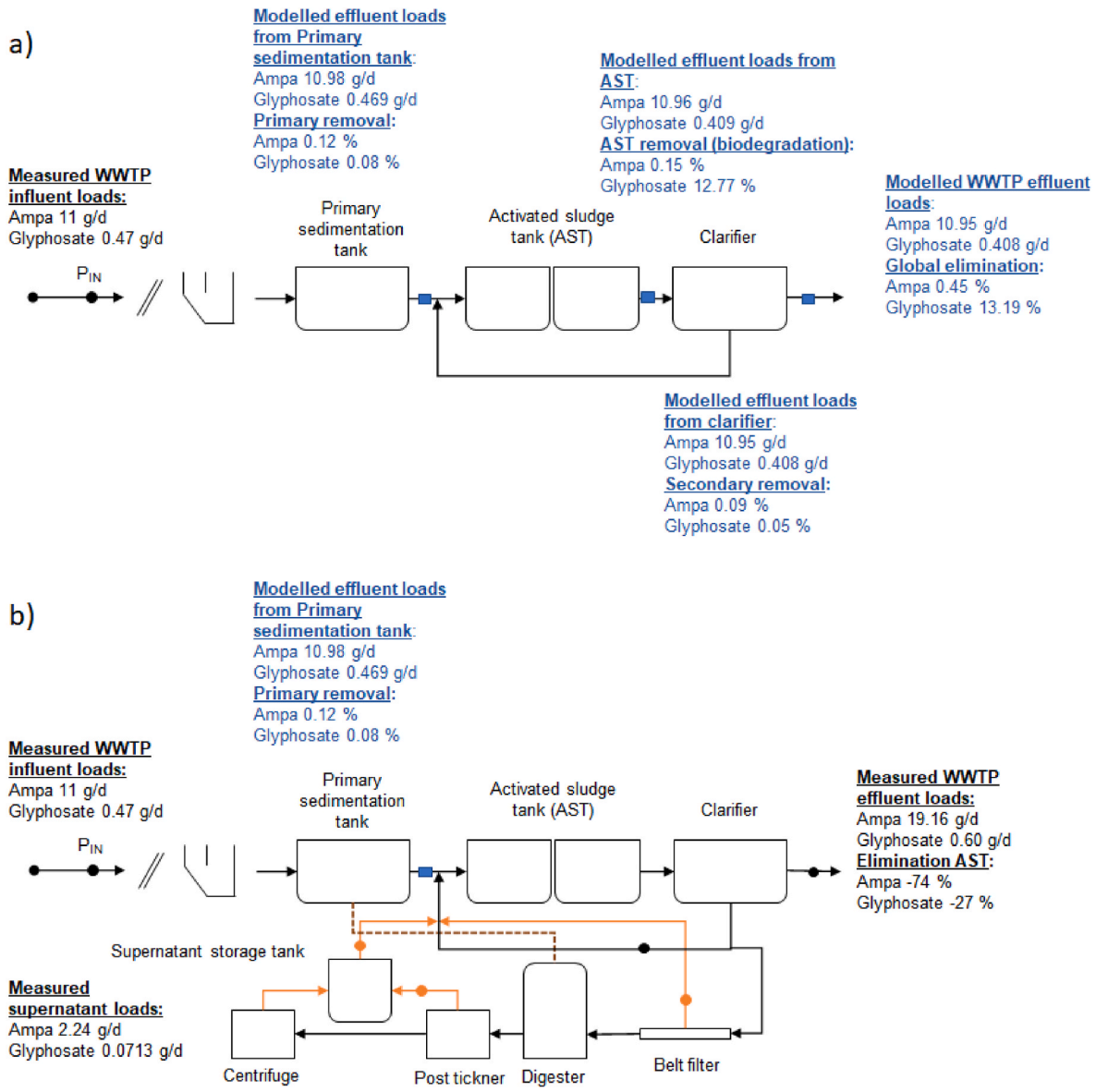

Fig. 4. Mass balance for AMPA and glyphosate (aqueous phase only) in a conventional activated sludge process (a) versus one equipped with a digester (b): ■ modelled concentrations with SimpleTreat, ● measured samples.

removal from Primary sedimentation tank and clarifier can be considered negligible while the compounds are expected to be released almost unchanged (only 12.77% of glyphosate is expected to be removed by biodegradation) and thus not removed in the WWTP effluent. When modelled loads are compared with measured load (Fig. 4, b), elimination of AST can be related to the additional load measured in the supernatant, which is daily reinjected in the AST.

According to the operator of the WWTP, at the least 23 $m^3\ d^{-1}$ of supernatant is daily returned to the activated sludge tank which results to 20 and 15% the daily average influent load of AMPA and glyphosate with 2.24 and 0.0713 g/d respectively.

The volume returned to the activated sludge can reach up to 3120 $m^3\ d^{-1}$. Following Equation (4), the load of glyphosate accumulated or biodegraded in the AST resulted to be around 0.541 g/d (0.47 g/d + 0.0713 g/d) and balance almost neutral. The biodegraded glyphosate can be converted into AMPA in a ratio that depends from different factors such as sludge retention time, pH and composition of microbial community.

3.3. Fate of AMPA and glyphosate within the post-treatment step

Intermittent regime was adopted for the operation of VF1 and VF2 because of the improved oxidation-reduction conditions favourable for complete nitrification and elimination of micropollutants, and the reduced clogging. The overall treatment efficiencies of VF1 and VF2 with respect to glyphosate and AMPA accounted for 87 and 38% during the observation period.

When looking at the average removal efficiencies (Figure S4 Supplementary information) calculated for each scenario, the effect of doubling load and operating the wetlands in parallel or alternating mode can be evaluated.

Results showed a non-significant difference in glyphosate removal between low and high hydraulic load. For AMPA the increase of load does not seem to be beneficial but high standard deviation of these compounds are reflected in their variability, which do not allow to give substantial conclusions. Overall, a hydraulic load of 200 $L\ m^{-2}\ d^{-1}$ could thus be adopted. Higher acceptable hydraulic load results in fact in a smaller required surface area.

The wetland performance was compared between alternating and

parallel mode at the same hydraulic load. Results did not show differences that can be significantly appreciated and suggested that both modes could be adopted.

However, looking at the variation of elimination along the operation period per single scenario and compound, few differences can be appreciated. Generally, the increase of load implies a consistent reduction of the removal rate.

Considering that the individual compound kinetic is expected to follow a pseudo-first order approximation along the operation time, the exponential distribution of each compound with respect to the load applied has been determined by curve fitting so that the results can easily compared (Fig. 5). While is almost negligible for glyphosate, the influence of the load negatively influenced the elimination of AMPA over all operation time.

The behaviour of OPPs in a constructed wetland environment can be anticipated looking at their physical properties. Compounds with high K_{oc} (values above 1000 mg L^{-1} and $LogK_{oc}>3$) are expected to be strongly adsorbed to the soil particles. Both glyphosate and AMPA present high K_{oc} values but a polar and hydrophilic character (low Log_{OW}, low pK_a) which would make them more susceptible to mineralization/degradation. Their high-water solubility increases the risk of being transported in the aqueous phase without being retained by the soil.

Several studies (Liu et al., 2019; Braschi et al., 2022) demonstrated the strong ability of glyphosate to adsorb to soil particles in a wetland environment depending on the characteristics of the soil. Because glyphosate presents an amine, carboxylate, and phosphonate group, it can react both as an acid and as a base with four associated constants, pK_a. Thus, its affinity for adsorption to soil particles can be related to the content of clay if complexed via cation exchange, iron and aluminium oxides in the soil together with its pH (negative relation). High adsorption can be expected with VF1 and VF2 considering that the Sand used in this study contains 55% SiO_2, 24% Al_2O_3, 14% Fe_2O_3 and 5% CaO.

The cumulative curves (Supplementary information, S.4) finally allow to quantify the amount of AMPA and glyphosate retained by the soil of the wetland at the end of the operation time, when a different load is applied under the hypothesis that the driving removal mechanism is adsorption. After 120 days of operation, the wetland has been able to adsorb around 9 and 12 μg L^{-1} when 100 and 200 L m^{-2} d^{-1} is applied respectively. Similarly for glyphosate but with much lower magnitude, 0.6 and 1.7 μg L^{-1} when 100 and 200 L m^{-2} d^{-1} is applied respectively. This suggest that even if the average removal does not show significative differences or appears negatively impacted, a higher amount of active ingredient is expected to be adsorbed when higher load is applied. Previous studies (Aparicio et al., 2013; Okada et al., 2016) showed evidence that sorption in soils facilitates microbial degradation and that sometimes adsorbed glyphosate can undergo carboxylation by microorganisms leaving AMPA as its transformation product. This suggests not only precaution in considering adsorption the main degradation pathway of glyphosate in a constructed wetland environment but also the possibility of generating AMPA as degradation product in soil especially under oxic conditions.

Same approach is used when the wetland performances are compared with respect to the operation mode (Fig. 6) evaluated for 200 L m^{-2} d^{-1}, with a late slightly better removal for parallel mode operation probably due to the longer rest time experienced from the soil which is beneficial for those compounds that are known to have long half-life time. High concentrations of AMPA in VF effluents generally confirm that the rate of AMPA degradation in soil is slower than that one of glyphosate. AMPA's typical half-life (DT50) in soil was established at 234 days against the 24.8 of glyphosate which suggests AMPA more persistent than glyphosate in wetlands.

Looking at the cumulative curves (Supplementary information, S.4), after 120 days of operation around 1.7 μg L^{-1} of glyphosate is adsorbed by the soil independently from the applied mode, while 10 and 12 μg AMPA L^{-1} when parallel and alternating mode is applied respectively.

The presence of AMPA and glyphosate was finally detected in the receiving waters (Sûre river) prior and during the duration of the investigation for a total of 38 water samples collected from the water administration over three years. Glyphosate concentrations ranged between 28 and 80 ng L^{-1} (median concentration 49 ng L^{-1}) while AMPA between 58 and 430 ng L^{-1} (median concentration 239 ng L^{-1}). However, measurements of particulate matter and sediments were neglected. The average WWTP effluent concentration of Glyphosate and AMPA were 2.5 and 17 times higher than those measured in surface water indicating the strong impact of urban agglomeration in the discharge of AMPA. This factor is reduced by 35% when a CW in VF configuration is applied.

4. Conclusions, recommendation, and perspective

- Non-agricultural application of glyphosate and/or transport of glyphosate to sewer systems via runoff is relevant in the annual load.

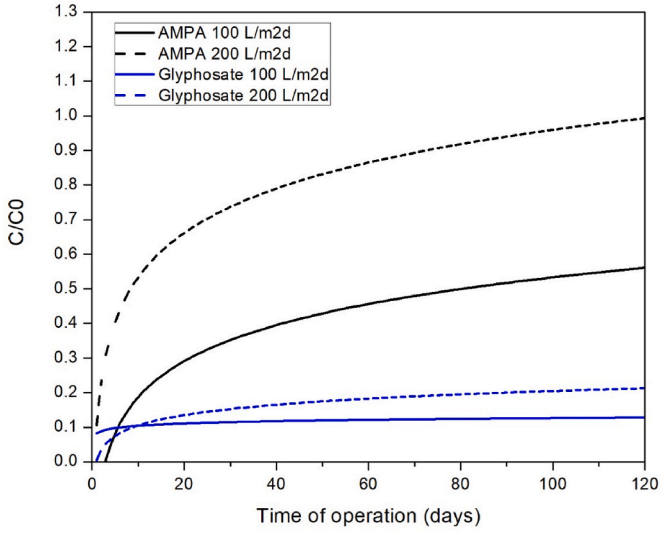

Fig. 5. AMPA and glyphosate removal of VF (operating in alternating mode) when higher load is applied.

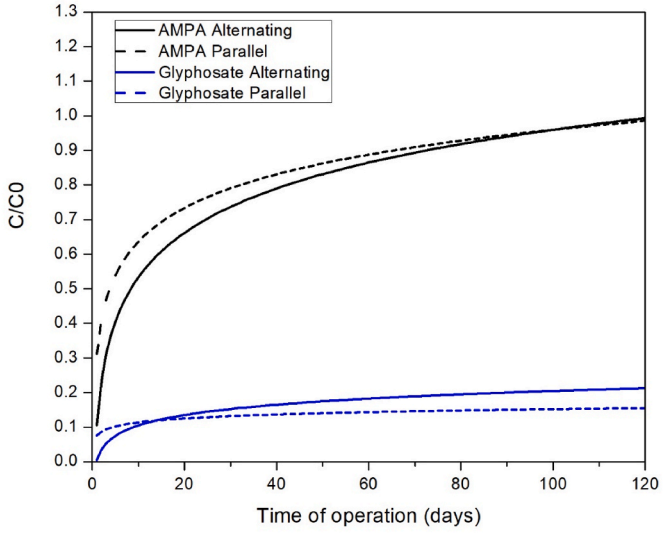

Fig. 6. AMPA and glyphosate removal of VF when alternating and parallel mode are compared.

- The origin of AMPA in the Sûre catchment is particularly related to the industrial stream contribution as photodegradation product of amino-polyphosphonates rather than as metabolite of glyphosate.
- The daily reject water has impact on the mass balance of both glyphosate and AMPA and can partially explain the negative removal during aerobic degradation. Future research should better investigate the fate of micropollutants in the anaerobic digester and their presence in the reject water. Both solid and liquid phases should be analysed according to a fractionated approach. This is particularly important for mass balance but also in the perspective of reuse or treatment of the reject water as for the understanding of the consequences of digested sludge disposal.
- CW applied as VF configuration can ensure almost 90% elimination for glyphosate while AMPA confirmed to have degradation in soil lower than glyphosate and being sensitive to higher hydraulic load rather than mode of operation.

Credit author statement

Silvia Venditti: Writing – original draft preparation, writing – reviewing and editing, Formal analysis, Methodology, Conceptualization, Investigation, Visualization, Data curation. Anne Kiesch: writing – reviewing and editing, Formal analysis, Methodology, Conceptualization, Investigation, Visualization, Data curation. Joachim Hansen: Supervision, Funding acquisition, Project administration, Writing – original draft preparation, writing – reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.139843.

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