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Review

A review of emerging organic contaminants (EOCs), antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in the environment: Increasing removal with wetlands and reducing environmental impacts

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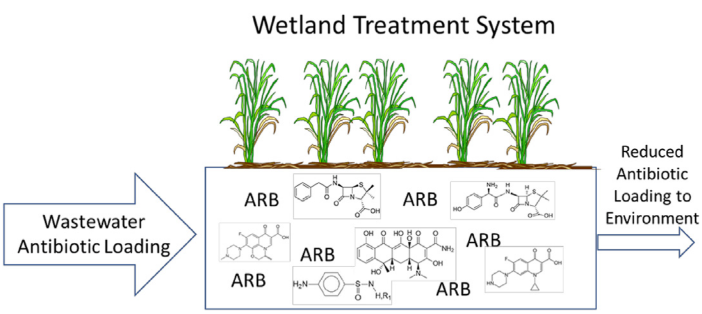
GRAPHICAL ABSTRACT



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ABSTRACT

Emerging organic contaminants (EOCs) include a diverse group of chemical compounds, such as pharmaceuticals and personal care products (PPCPs), pesticides, hormones, surfactants, flame retardants and plasticizers. Many of these compounds are not significantly removed in conventional wastewater treatment plants and are discharged to the environment, presenting an increasing threat to both humans and natural ecosystems. Recently, antibiotics have received considerable attention due to growing microbial antibiotic-resistance in the environment. Constructed wetlands (CWs) have proven effective in removing many EOCs, including different antibiotics, before discharge of treated wastewater into the environment. Wastewater treatment systems that couple conventional treatment plants with constructed and natural wetlands offer a strategy to remove EOCs and reduce antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) far more efficiently than conventional treatment alone. This review presents as overview of the current knowledge on the efficiency of different wetland systems in reducing EOCs and antibiotic resistance.

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1. Introduction

In recent years there has been growing concern about the release of organic compounds of anthropogenic origin, known as emerging organic contaminants (EOCs), to the environment. These EOCs include a diverse group of thousands of chemical compounds, such as pharmaceuticals and personal care products (PPCPs), pesticides, hormones, surfactants, flame retardants, plasticizers and industrial additives, among others. Metabolites and intermediate degradation products of parent compounds are also included (Farré et al., 2008). The ubiquity of EOCs in the environment poses a threat to many non-target living organisms since they are designed to remain biologically active for long periods. The presence of antibiotics is of special concern due to the development of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs). These substances are extensively used in both human and veterinary medicine against microbial infections and are excreted from the body of the treated organisms, together with their metabolties, within a few days of consumption. It has been widely demonstrated that conventional sewage treatment plants (STPs) are inefficient in the removal of many PPCPs, including antibiotics, ARBs and ARGs, thus contaminanting the receiving ecosystems with a complex mixture of bioactive agents and bacteria (Cacace et al., 2019; Corno et al., 2019; Manaia et al., 2018).

Once in the environment, antibiotics can lead to the continuous selection for ARB that contain ARGs (Choo, 1994; Costanzo et al., 2005; White et al., 2006: Ávila and García, 2015; Sui et al., 2015; Shiffett and Schubauer-Berigan, 2019). Although ARB are a threat to public health, ARGs are the underlying mechanism of an increasing antibiotic tolerant microbial consortia. In recent years, medical professionals and scientists globally have become concerned over the prevalence of ARGs and ARB that have appeared as the result of over prescription/production of antibiotics. Overuse of antibiotics can range from doctors prescribing them ineffectively to patients for viral infections (Gonzales et al., 1997), patients using other people's antibiotics or old antibiotics and the use of antibiotics as growth promotors and feed additives in livestock and poultry (Kim and Aga, 2007). In 2019, antibiotic-resistant bacteria and fungus caused more than 2.8 million infections and 35,000 deaths in the United States alone (CDC, 2019). Currently, there are approximately 260 different antibiotics in about 20 different families or classes (Everage et al., 2014). A focus of this paper is to review how constructed and natural weltands can enhance removal of antibiotics.

In this paper, we review the occurrence and impact of EOCs, especially PPCPs, ARB and ARGs in the environment and address the potential of wetlands to remove these compounds from wastewaters. For PPCPs, we focus on antibiotics which are the main cause of ARB and ARGs. There are already several reviews on the occurrence of PPCPs in the environment and removal by constructed wetlands (García et al., 2010; Zhang et al., 2014), but few specifically focused on ARB and ARGs (Liu et al., 2019). The main objective of this paper is to discuss the prevalence of PPCPs, ARB, and ARGs in aquatic ecosystems and the mechanisms of removal using constructed and natural wetlands.

2. Sources of PPCPs, antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs)

After intake, antibiotics rarely become fully metabolized in the body and thus are partially excreted in their original form (Zhang et al., 2009). Together with their metabolites, they are excreted from the body through urine and feces within a few days of consumption. In rural areas, direct excretion from medicated cattle in animal husbandry facilities is the main entrance route into the environment, together with manure application as fertilization amendments (considering also biosolids from STPs), farm runoff (Bird et al., 2019; Chee-Sanford et al., 2009; Dolliver and Gupta, 2008; Sabourin et al., 2009) and fish farming (Sapkota et al., 2008). In urban areas, the regular discharges of STP effluents into aquatic bodies (e.g. rivers, lakes), including hospital wastewater effluents, is the main entrance pathway of these substances into the environment (Hocquet et al., 2016; Rodríguez-Mozaz et al., 2015; Michael et al., 2013). It is estimated that 30–60% of all prescribed antibiotics can end up in STPs, which act as primary reactors creating ARB and ARGs (Costanzo et al., 2005). Recent studies show that incomplete metabolism in humans and improper disposal of antibiotics to sewage treatment plants (STPs) has been a main source of antibiotic release into the environment (Everage et al., 2014; Naquin et al., 2015; Boopathy, 2017; Grabert et al., 2018).

There are many commonly prescribed antibiotics that are found in ng to µg L^{-1} concentration levels in sewage wastewater and treated effluents (Grabert et al., 2018; Bird et al., 2019). These antibiotics exert constant selective pressure on microbial populations in developing antibiotic resistance. Conventional STPs, based on activated sludge (CAS) biological treatment, are an ideal place for the development of ARB and ARGs as the sewage is concentrated in the activated sludge process and the microbes are actively multiplying with constant exposure to low concentrations of a multitude of antibiotic drug types. This mixture of differentially acting drugs and high microbial activity becomes an ideal environment for adaptive evolution of antibiotic resistance in microbes. There are various reports on the occurrence of ARGs in treated municipal wastewater effluents worldwide. Tables 1 and 2 summarize a representative selection of the genes reported, but there are numerous other studies not included here because of the presence of the same genes in different geographical locations.

3. Antibiotic resistance acquisition

It is important to understand that antibiotic resistance can be present in all bacteria, not solely pathogenic bacteria (Hawkey, 1998). Bacteria often have two distinct types of resistance to antibiotics, intrinsic and acquired resistance. Intrinsic resistance is a naturally occurring trait in the organism, while acquired resistance is the evolution of sensitive bacteria to resistant bacteria (Hawkey, 1998). Organisms most often develop resistance to antibiotics because of spontaneous mutations in their DNA structure, regardless of the amount of antibiotics actually present.

Bacteria with resistance to antibiotics started to appear soon after antibiotic use became widespread (Bronzwaer et al., 2002). Although

Table 1
ARGs for sulfonamide and tetracycline reported in the sewage treatment plant effluents.

Reported Gene	Sewage Plant Location	Reference
Sulfonamide Resistance Genes		
Sul-1	Michigan, USA	Munir et al., 2011
Sul-1	Lausanne, Switzerland	Czekalski et al., 2012
Sul-II	Lausanne, Switzerland	Czekalski et al., 2012
Sul-1	Minnesota, USA	Burch et al., 2013
Sul-1	Shafdan, Israel	Negreanu et al., 2012
Sul-1I	Shafdan, Israel	Negreanu et al., 2012
Sul-1	Hangzhou, China	Chen & Zhang, 2013
Sul-1I	Hangzhou, China	Chen & Zhang, 2013
Sul-I	Louisiana, USA	Naquin et al., 2015
Tetracycline Resistance Genes		
tetW	Michigan, USA	Munir et al., 2011
tetO	Michigan, USA	Munir et al., 2011
TetQ	Wisconsin, USA	Auerbach et al., 2007
tetG	Wisconsin, USA	Auerbach et al., 2007
tetC	Hong Kong, China	Zhang et al., 2009
tetA	Nanjing, China	Zhang et al., 2009
tetA	Minnesota, USA	Burch et al., 2013
tetW	Minnesota, USA	Burch et al., 2013
tetX	Minnesota, USA	Burch et al., 2013
tetM	Hangzhou, China	Chen & Zhang, 2013
tetX	Louisiana, USA	Naquin et al., 2015
tetA	Louisiana, USA	Bird et al. 2018
tetX	Louisiana, USA	Belding&Boopathy, 2018.

Table 2
ARGs for Beta-Lactam and macrolide sulfonamide and tetracycline reported in sewage treatment plant effluents.

Reported Gene	Sewage Plant Location	Reference
Beta-Lactam		
$Bla_{TEM-uni}$	Massachusetts, USA	Lachmayr et al., 2009
blaM-1	South Carolina, USA	Uyaguari et al., 2011
Bla_{-vim}	Germany	Schwartz et al., 2003
amp-C	Germany	Schwartz et al., 2003
amp-C	Spain, Italy, Belgium	Bockelmann et al., 2009
$Blas_{hv-5}$	Spain, Italy, Belgium	Bockelmann et al., 2009
mecA	Gothenburg, Sweden	Borjesson et al., 2009
mecA	Louisiana, USA	Boopathy, 2017
mecA	Louisiana, USA	Naquin et al., 2015
Macrolide Resistance Genes		
ermB	Minnesota, USA	Burch et al., 2013
ermF	Shafdan, Israel	Negreanu et al., 2012
ermB	Shafdan, Israel	Negreanu et al., 2012
ermB	pain, Italy, Belgium	Bockelmann et al., 2009
Others		
VanA	Spain, Italy, Belgium	Bockelmann et al., 2009
VanA	Germany	Schwartz et al., 2003

scientists noted the phenomenon of resistance as early as the mid-twentieth century, it is now understood that antibiotic resistance has likely existed for as long as microorganisms have been able to produce antibiotics naturally. Because antibiotics remove all the susceptible bacteria from the treated individuals, the only bacteria left are those resistant to the treatment, which may flourish and spread resistance throughout the environment. Multi-drug resistant bacteria have been cultured from habitats isolated from anthropogenic disturbances for hundreds of years (Bhullar et al., 2012). It is expected that in the near future many bacteria will evolve to become completely resistant to most or all antibiotics used today that will increase risks to the environment and to humans.

Bacteria are able to acquire ARGs through mutations and through gene transfer. The main method of antibiotic gene transfer is through horizontal gene transfer (Salyers et al., 2004). In this method, bacteria acquire the genes from other bacteria within and across different species. Every time bacteria multiply and divide, the ARGs spread vertically in an exponential scale, enabling this rapid spread also in the aquatic environment. Depending on the antibiotic, many different genes may exist that allow the bacteria to survive to antibiotic exposure. For example, 38 different tetracycline resistance genes have been found and described in bacteria (Roberts, 2005; Grabert et al., 2018).

4. PPCPs, ARB and ARGs in the environment and their impacts

The continued use of antibiotics is likely to increase the frequency of antibiotic resistance in the environment (Gillings and Stokes, 2012). For instance, soil samples in the Netherlands were shown to contain up to 15 times more genes-encoding resistance in 2008 when compared to soil samples from 1970 (Knapp et al., 2010). Furthermore, antibiotics can survive for extended periods of time in the environment and free DNA carrying ARGs can last up to two years in the soil.

Urban wetlands may become reservoirs of ARGs, particularly where wastewater is improperly managed or treated. A clear example of this is demonstrated in wetlands associated with the Tijuana River that discharges to the Pacific Ocean near the US-Mexico border (Cummings et al., 2010). This river receives drainage from the Tijuana metropolitan area including inadequately treated sewage and landfill runoff and stormwater. Cummings et al. (2010) analyzed surface sediments from the river estuary and a nearby urban wetland, Famosa Slough, that is largely unaffected directly by sewage. They found plasmid-mediated quinolone resistance genes *qnrA, qnrB, qnrS, qepA, and aac(6′)- Ib-cr* in the Tijuana River Estuary, and only *qnrB, qnrS, and qepA* at Famosa Slough.

Belding and Boopathy (2018) examined natural surface waters near Chauvin and Port Fourchon, Louisiana, in the lower Mississippi River Delta, and found significant numbers of ARB and ARGs, indicating their widespread presence in coastal waters used for recreation and fishing. Bird et al. (2019) also identified ARB and ARGs in Bayou Lafourche, an important waterway in southeastern Louisiana. In addition, fecal coliforms were consistently higher than water quality standards. In this case, the primary cause for this contamination was related to livestock, which was heavily treated with antibiotics that later were released into the environment in animal feces and urine. Although the antibiotics were found in small concentrations, antibiotics at sublethal concentrations provided the selection pressure needed to promote genetic exchange leading to ARB and ARGs without killing the entire microbial pool. The Louisiana Department of Environmental Quality reported that failing septic systems and runoff from residential areas were also significantly contributing to the presence of fecal coliforms in Bayou Lafourche (LDEQ, 2018; Martinez et al., 2019). An estimated 1.3 million Louisiana residents treat and dispose of sewage using on-site septic systems that are not connected to municipal treatment systems, of which an estimated 50% may be failing or malfunctioning. Individual septic tanks contribute significantly to the increase and spread of ARGs due to the low level of treatment and lack of any significant disinfection. They also reported ARB and ARGs in the Mississippi River which receives treated wastewater and agricultural runoff from a large expanse of the central United States (Bird et al., 2019).

5. Efficiency of conventional wastewater treatment on PPCPs, ARB and ARGs removal

Secondary municipal wastewater treatment using conventional activated sludge (CAS) does not substantially remove PPCPs and removal rates are highly compound specific (Conkle et al., 2008; Ávila and García, 2015; García et al., 2010; Zhang et al., 2014). Removal mechanisms during CAS treatment include microbial degradation and sorption to particulate matter (Conkle et al, 2010). However, sludge can retain significant concentrations of PPCPs that can be released back into the environment after biosolids application (Chen et al., 2016a,b). In addition, the hydraulic retention time (HRT) in CAS treatment is short, often less than 12 h, and is not adequate for PPCPs removal.

In addition, STPs can increase ARB and ARGs in wastewater during the treatment process. In a municipal treatment system in south Louisiana, Boopathy (2017) reported that raw sewage as well as secondary effluent from a treatment plant tested positive for Methicillin-Resistant *Staphylococcus aureus* (MRSA), and free DNA of *mecA* gene. The antibiotic resistance was significantly higher in treated sewage than in raw sewage, indicating that bacteria were acquiring ARGs during the treatment process.

There are a number of advanced tertiary treatment technologies that can effectively remove PPCPs such as ozonation, advanced chemical oxidation or ultraviolet (UV) radiation, but these approaches are generally very expensive, especially for small municipalities (White et al., 2006). For these reasons, Ávila and García (2015) suggested the use of decentralized, low-cost technologies including constructed and natural wetland treatment for small municipalities. Wetlands are less expensive and have lower maintenance costs than highly engineered systems, and can tolerate fluctuations in daily flow rate and load, a signature of wastewater production (Zurita et al., 2012). Wetlands have also been shown to remove many PPCPs (White et al., 2006; Conkle et al., 2008; Chen et al., 2016c; Fang et al., 2017; Dires et al., 2018; Hayward et al., 2018; Santos et al., 2019). Constructed wetlands (CWs) are more land intensive and require from 2 to 8 m^2/PE (person equivalent) compared to 0.06 m^2/PE for conventional treatment and about 30 m^2/PE for natural assimilation wetlands (Fig. 1) (White et al., 2006). This large land requirement favors wetland treatment for

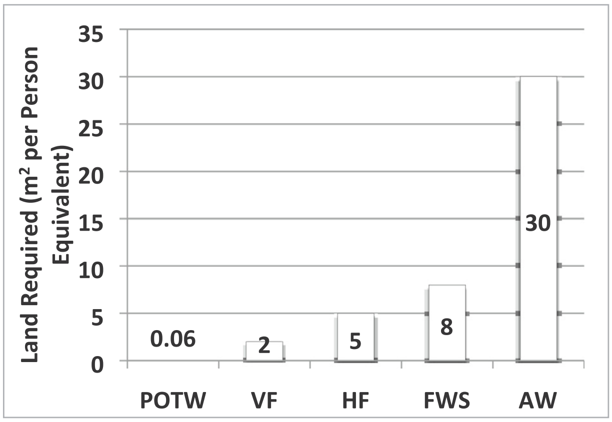

Fig. 1. Land requirements (m^2 per person equivalent per 6500 m^3/day) for publicly-owned treatment works (POTW), vertical flow constructed wetlands (HF), horizontal flow constructed wetlands (HF), free water surface wetlands (FWS), and assimilation wetlands (AW). Values fro POTW, VF, HF, and FWS from Ávila and García (2015). Value for AW calculated from Conkle et al. (2008).

medium to small communities as larger urban centers typically do not have the open space for large-scale wetland treatment. To overcome this limitation, the City of Orlando, Florida sends their treated wastewater over 20 km through underground piping to Christmas, Florida, a more sparsely populated community with land available for wetland treatment (Wang et al, 2006; White et al, 2006).

6. Removal of EOCs, ARB and ARGs in constructed wetlands

Constructed wetlands (CWs) for wastewater treatment are a state of the art technology with thousands of full-scale applications at a global scale. CWs are being increasingly used for decentralized wastewater treatment due to their simple design and because operation and maintenance costs are typically much lower than conventional systems. Wetlands do not require any chemical addition and their sludge production is negligible (Álvarez et al., 2017; Paing et al., 2015). Efficiency of CWs for the improvement of conventional water quality has been widely evaluated and demonstrated over many decades, and very positive results have also been obtained regarding EOCs removal (Ávila et al., 2016; Ávila and García, 2015; Conkle et al., 2008; Verlicchi and Zambello, 2014).

Constructed wetlands are man-made wetlands that are classified based on how water flows through the wetland (i.e., surface flow and subsurface flow). In general, subsurface flow systems (SSF) are used in the framework of decentralized sanitation, while surface flow systems are usually larger in scale than subsurface flow wetlands, and are frequently used as tertiary treatment. Recent studies on CWs efficiency have shown the capacity of these systems to remove not only PPCPs, but also ARB and ARGs from wastewaters (Chen et al., 2019; Liu et al., 2019). The direct discharge of STP effluent to some natural wetlands also results in significant removal of these compounds (White et al., 2006: Conkle et al., 2008).

The main removal mechanisms in CWs include biodegradation, substrate adsorption, precipitation, plant uptake, photolysis and hydrolysis (García et al., 2003; Uggetti et al., 2016; Ávila et al., 2017; Álvarez et al., 2017; Pelissari et al., 2018). Because of the diversity of EOCs and ARB the removal mechnisms will vary based on the EOCs chemical properties (Liu et al., 2019). Most of the CWs studied achieved desirable PPCPs, antibiotic and ARGs removals, and outperformed conventional wastewater treatment systems (Chen and Zhang, 2013; Chen et al., 2016c; Hijosa-Valsero et al., 2010; Xu et al., 2015; Zhang et al., 2014). For instance, detectable concentrations of ARGs (*tetA, tetM,* and *ampC*) in municipal wastewater were removed in a HSSF-CWs mesocosm experiment after 150 days of treatment (Nõlvak et al., 2013). These authors observed a clear correlation between the high removal of NH_4–N and organic matter and the reduction of ARGs in the CW effluent.

Matamoros and Bayona (2006) evaluated the removal of 11 PPCPs in a decentralized sanitation plant in Spain, consisting of 2 parallel HSSF-CWs with different water depths, both planted with *Phragmites australis*. Removal was generally greater in the shallower bed due to a higher passive aeration and a less negative redox potential that enhanced biodegradation of the compounds. The most hydrophobic compounds (with higher K_d), such as the musks tonalide and galaxolide, had 80% removal from the aqueous fraction and were retained in the gravel of the filter bed. Only the non-steroideal anti-inflammatories diclofenac and ketoprofen were not efficiently removed in both systems, due to their high hydrophobicity and low biodegradation potential. The recalcitrance of these two compounds has been widely demonstrated in conventional STPs (Gros et al., 2010, 2007; Mamo et al., 2018). In another work by the same authors, the removal of 13 PPCPs was studied in a variety of field scale, onsite confined domestic wastewater treatment systems in Denmark, including biological sand filters, compact biofilters, VSSF-CWs and HSSF-CWs serving from 2 to 280 population equivalents (Matamoros et al., 2009). The removal rate for PPCPs was generally > 80% with the exception again of diclofenac and ketoprofen, and the antiepileptic drug carbamazepine. Carbamazepine was also poorly removed in a surface flow wetland system in LA (Conkle et al., 2008). Despite the overall high removal rates, the vegetated VSSF-CWs, with greater oxygenation in the unsaturated gravel filter yielded better removals. White et al. (2006) reported the complete removal of five out of nine PPCPs occurring in a sewage effluent pumped to a large treatment wetland in Orlando, FL, but again carbamazepine was not efficielty removed.

Liu et al. (2019) reviewed the capacity of 106 different CW treatment systems to eliminate 39 antibiotics, including sulfonamides, quinolones, tetracyclines, macrolactones, chloramphenicol, polyethers and beta-lactams. CW configurations included surface flow wetlands (SF-CWs), HSSF-CWs, VSSF-CWs and hybrid flow constructed wetlands (HyF-CWs), all at microcosm-scale or mesocosm-scale. VSSF-CWs were the most effective in eliminating antibiotics (> 70% for most of the antibiotics), and these results were significantly different (p less than 0.05) from the other types of CWs (Fig. 2). It should be noted that the systems compared in this study operated under very different conditions (solar radiation, temperature, size, influent quality, etc.). Nevertheless, this review confirmed the crucial role of the wetland configuration in the overall removal efficiency of the system, as confirmed by a number of other studies (Chen et al., 2016c; Hijosa-Valsero et al., 2011; Huang et al., 2017, 2015).

VSSF-CWs are generally more efficient than HSSF-CWs in the removal of organic matter, ammonia and also EOCs. Intermittent discharge in VSSF-CWs creates unsaturated conditions between pulses, promoting aeration/oxygenation of the filter medium during those intervals (Kahl et al., 2017; Nivala et al., 2019). Nitrification capacity is greatly enhanced by these intermittent, unsaturated conditions (García et al., 2010), which can also enhance the degradation of different antibiotics by co-metabolism (He et al., 2018; Kassotaki et al., 2016; Müller et al., 2013). In contrast, in HSSF-CWs the granular medium remains continously saturated and still, positive results have been obtained for these systems. Nõlvak et al. (2013) demonstrated that detectable concentrations of ARGs (*tetA, tetM* and *ampC*) in municipal wastewater influents were removed in a HSSF-CWs mesocosm experiment after 150 days of treatment, with a clear correlation between the high removal of NH_4–N and organic matter and the reduction of ARGs in the CW effluent.

A relatively new configuration of VSSF-CW, in which the higher part of the filter media is only partially saturated, enhances a variety of redox gradients through the filter bed and also diversifies the bacterial metabolism developed in the system, improving nitrogen

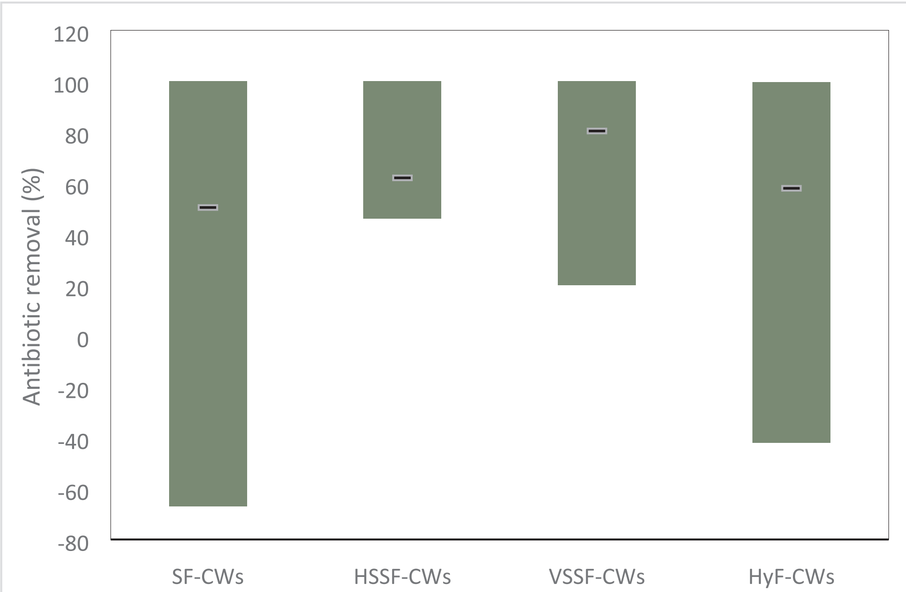

Fig. 2. Antibiotic removal efficiencies of different constructed wetlands (average values). SF-CWs: surface flow constructed wetlands (n = 31); horizontal subsurface flow constructed wetland (HSSF-CWs) (n = 24); vertical subsurface flow constructed wetlands (VSSF-CWs) (n = 31); hybrid flow constructed wetlands (HyF-CWs) (n = 20). The different studies were carried out at a microcosm-scale or mesocosm-scale (Liu et al., 2019).

transformation pathways and achieving complete nitrification-denitrification (Ávila et al., 2017; Dong and Sun, 2007; Pelissari et al., 2018, Pelissari et al., 2017). This partial saturation has already achieved higher removal rates of different EOCs including antibiotics such as trimethoprim and sulfamethoxazole (Sgroi et al., 2018). All in all, subsurface flow CWs are more efficient in activating and removing ARGs than surface flow CWs (Chen et al., 2016b; Decamp and Warren, 2001). These findings are in agreement with previous research on organic matter and nutrient removal in CWs (Carvalho et al., 2014; Vymazal, 2011). This may be due to a greater exposure to particles of the granular mediun resulting in higher sorption capacity and more complex biological processes occurring in the filter media of subsurface flow CWs with close proximity of aerobic and anaerobic zones. However, a clear correlation has not been established yet between redox mediated biological processes and ARGs removal, and this is a topic that deserves intensive research.

The removal efficiency of different antibiotic families in CWs varies with the physical characteristics (e.g., water solubility, octanol-water distribution coefficient (K_{ow})) of the componds. Those compounds with the best removal rates (e.g., quinolones and tetracyclines at 70–90%) generally have a low solubility, a high tendency to adsorb to gravel in the filter media, and are prone to photodegradation (Jia et al., 2012). For instance, high K_{ow} values and low water solubilities, as in the case of macrolides, lead to high adsorption by plants and the filter media (Liu et al., 2019). Average removal efficiencies of macrolides were high in VSSF-CWs (81.73%). In contrast, sulfonamides are highly polar substances, which makes them highly mobile once in the environment, being frequently found in the water (they are also generally soluble). The Log P for most of them < 0. Microbial degradation plays a key role in the reduction of sulfonamide concentration in the wastewater. Average eliminations for sulfonamides ranged from 13% to 99.9%. Among the different antibiotics studied, sulfamethoxazole and erythromycin have raised scientific concern due to their high concentrations and frequencies of detection in feed waters to CWs, and the low reduction rates obtained (Liu et al., 2018; Yang et al., 2018). Conkle et al. (2008) demonstrated the strong sorption to soil particles of three fluoroquinolone antibiotics individually. However, when mixtures of the 3 antibiotics were applied, there was substantially less sorption of two of the compounds, clearly indicating a competitive sorption among compounds that sorb well alone. Considering how many different antibiotics can be in a particular waste stream, sorption behavior in mixtures needs to be further evaluated.

On the other hand, different studies have reported negative removals of antibiotics in CWs, meaning that higher concentrations were detected in the effluents of the systems than in the corresponding influents (Fang et al., 2017; Conkle et al, 2010). These results are not infrequent either in conventional STPs, and are usually related to the presence of second-phase metabolites in influent wastewaters, such as acetylates or glucuronides. These metabolites, which are not considered or measured in most of the studies, can deconjugate and transform back into the parent compound (García-Galán et al., 2012; Kasprzyk-Hordern et al., 2009), thus accounting for the higher concentrations observed in the CWs effluents than in the influent to the systems. For more hydrophobic antibiotics, which are adsorbed and retained in the filter media of the CWs, their eventual release has also been confirmed, contributing also to these negative removal rates (Li et al., 2014). Indeed, solid particles tend to act as a reservoir for ARB and ARGs in CWs, thus a pulsing regime that allows the systems to "rest" (VSSF-CWs) will favor continued reduction in PPCPs, ARB and ARGs. Fang et al. (2017) observed that the concentration of ARGs increased in the soil of an HyF-CW consisting of 3 SF-CWs connected in series and planted with different macrophytes. This facility has been operated for 10 years, receiving domestic sewage from a population of roughly 4000 people in Nanchang, (Jiangxi province, China). The authors evaluated ARGs and ARB removal efficiency, and observed that most of the ARGs detected in the aqueous phase were eliminated, but their levels increased in the solid phase. As a result, this phase could become an important source of aqueous ARGs through the desorption and release of microbes from soil to the water phase.

Hydraulic loading rate (HLR) and hydraulic retention time (HRT) are also key parameters in the CWs removal efficiencies of ARGs (Anderson et al., 2013; Maal-Bared et al., 2013; Miller et al., 2014). Longer HRT in CWs may lead to ARGs accumulation in the media which eventually can be discharged in the effluent. Seasonal variability in CWs retention efficiencies were also observed in the study by Fang et al. (2017) and absolute and relative abundances of ARGs in the CWs medium were higher during summer than winter, due mainly to warmer temperatures promoting the survival of bacterial communities in the soil and also larger communities of ARGs carriers. Higher HLR during summer may have also enhanced the diffusion and exchange of these ARGs carriers between the soil and water phase, due to a higher mechanical turbulence of the water phase. In VSSF-CWs, higher HLRs and infiltration rates compared to those in HSSF-CWs, would filter out bacteria (size exclusion) and bind extracellular DNA onto soil particles

(Cardinal et al., 2014). Longer HRT in the CWs has beneficial role of reducing EOC concentration as microbes in the CWs can interact long time with EOCs and thus reduce their concentration.

The type of filter media has also been studied and considered as a relevant determinant feature of CWs removal efficiency. For instance, different studies have demonstrated that antibiotics and ARGs removal in zeolite were higher than those of volcanic rock in VSSF-CWs (Liu et al., 2013) and also higher than those obtained in oyster shell, medical stone and ceramic media in HSSF-CWs (Chen et al., 2016c). Higher relative surface area, micropores and Si-OH structures for chemical sorption and microbial attachment improve the removal of ARGs (Hu et al., 2012). Dires et al. (2018, 2019) evaluated the effectiveness of broken brick as filtering material in 8 HSSF-CWs (mesocosms scale) to remove nutrients and ARB from hospital wastewater in Ethiopia. The treatment wetlands achieved 7.1 log10 and 5.1 log10 removal of total and fecal coliforms, respectively. Absolute abundances of ARB were reduced from the influent to the effluent by up to 93%.

Dires et al. (2018, 2019) also highlighted the role of vegetation in CWs, as they observed that ARB removals were higher in planted CWs (81%–93%) than in unplanted wetlands (42%–74%). Plants do not seem to be directly involved in the removal efficiency of ARGs, but they are involved indirectly. They are crucial in filtering solid particles and delivering small amounts of oxygen to microbial communities, which enhances the rhizhospheric bacteria and thus influencing ARGs abundance in some cases (Anderson et al., 2013; Chen and Zhang, 2013; Fang et al., 2017). On the other hand, they also provide the surface for biofilm development, which in turn enhances the microbial removal ability, and their subsequent harvesting in surface systems can reduce ARGs accumulation (Huang et al., 2015). Common reed (*Phragmites australis*) is widely used in CWs systems, and its presence has proved quite efficient in reducing ARGs and 16sRNA levels compared to other plants (Fang et al., 2017; Yi et al., 2017). Other aquatic plants such as *Pontederia cordata*, *Myriophyllum verticillatum* and *Cyperus alternifolius* can also contribute to removal of ARGs (Chen et al., 2015). Chen et al. (2016b) observed significant removal of antibiotics and ARGs in 6 different mesocosm-scale CWs, all of them with different flow configurations and plant species. As previously reported, SSF systems yielded better aqueous removal rates than SF systems, and the authors also observed that biodegradation was the main removal pathway, in terms of mass removal, compared to substrate adsorption or plant uptake. By contrast, Helt et al. (2012) found that adding ciprofloxacin to mesocosm-scale wetlands planted with *P. australis* led to an increase in resistance, not only to ciprofloxacin, but to other classes of antibiotics as well (cephalosporins, penicillins, tetracyclines, and sulfonamides), highlighting the potential for a single antibiotic exposure event to increase the antibiotic resistance profile to different antibiotics.

In another study by Chen and Zhang (2013), the fate of ARGs of *tetM*, *tetO*, *tetQ*, *tetW* (from tetracycline) *sulI*, *sulII* (sulfadiazine) *intI1*, and 16S rDNA genes was evaluated in a HSSF-CW, resulting in a reduction of 1–3 orders of magnitude of the ARGs initial concentrations. The authors concluded that the HSSF-CW could perform better than advanced treatments such as UV disinfection or biological aerated filters.

Solar radiation is important in SF-CWs as UV light alters DNA structures and can be lethal for bacteria. In the study by Fang et al. (2017), the removal of 14 ARGs was evaluated in a field-scale HyF-CW operating since 2005. The authors measured ARGs removal of 78% and 60% in winter and summer, respectively, demonstrating the influence of season on the performance of CWs, as higher temperatures may favor the activity of microbial communities and ARGs-containing microorganisms. The concentration of ARGs in the effluent was still 1 or 2 orders of magnitude higher than the levels in the influent stream. Indeed, concentrations of five ARG (*sul1, tetA, tetC, tetE, and qnrS)* in winter and of six ARGs *(sul1, sul3, tetA, tetC, tetE, and qnrS*) in summer increased throughout the treatment process. In contrast, Yi et al. (2017) measured high removal rates for several ARGs (*sulI*, *sulII*, *intI1*, qnrA) and 16 sRNA during a summer study in a 5.1 ha HSSF-CW and reported that the effluent from the CW had ARGs concentrations comparable to those of the receiving surface waters

All these studies demonstrate that coupling municipal wastewater treatment systems to wetlands for tertiary treatment yields higher removal of antibiotics as well as ARB and ARGs than conventional treatment systems alone. The removal of antibiotics is crucial to slow down the proliferation of ARB and ARGs in the environment, as their presence in wastewaters also favors the persistence of ARB and ARGs (Kümmerer, 2004).

7. PPCPs in small-scale domestic (septic) treatment systems

In many areas with a low-density population, such as suburban and semi-rural situations, sewage treatment for a significant proportion of the population is provided by small-scale (septic tanks) and package plant treatment systems. For example, over 85% of the population in some areas of the United States use septic systems (Godfrey et al., 2007) mainly because conventional centralized systems for such populations is very expensive (Matamoros et al., 2009). Coastal Louisiana is an example of an area with large numbers of septic systems. The southeastern part of the state is the most populous area in Louisiana, but only in the metropolitan areas of New Orleans and Baton Rouge are most of the population served by conventional STPs. In these two areas, a large proportion of treated municipal effluent is discharged to the Mississippi River. In the rest of that area, and in much of Louisiana, most effluent flows, directly or indirectly, into open water systems. Approximately 1.3 million residents in Louisiana treat and dispose of sewage on-site (not connected to municipal treatment) and an estimated 50% of these systems may be failing or malfunctioning (Fig. 3) (LDEQ, 2018; Martinez et al., 2019). This is similar in other areas in the US.

Septic systems and other on-site treatment systems such as package plants are also important sources of PPCPs (Sui et al., 2015). Most studies of small-scale onsite wastewater treatment systems include limited information on PPCPs in septic tanks and/or soil zones (Hinkle et al., 2005; Carrara et al., 2008; Huntsman et al., 2006). Swartz et al. (2006) monitored the concentrations of caffeine and its metabolite paraxanthine, as well as other micropollutants, in a residential septic system and downgradient groundwater. Concentrations of caffeine and paraxanthine were extremely high (caffeine ranged from 17,000 to 23,000 ng/L and paraxanthine from 55,000 to 65,000 ng/L) in the septic tank discharge, resulting in high concentrations of the compounds in the nearest well (> 1700 ng/L) that declined with distance and depth. A recent study concerning the occurrence and fate of different micropollutants in groundwater networks affected by septic systems showed that concentrations of several PPCPs such as carisoprodol (a muscle relaxant) and lidocaine (a typical anesthetic) could be greater than 0.1 μg/L in groundwater below and downgradient of the leaching bed (Phillips et al., 2006).

Considering that individual septic systems do a very poor job in removing EOCs from wastewater (Phillips et al., 2015; Swartz et al., 2006), the high number of septic systems and other decentralized treatment systems in the US pose a serious threat to the aquatic ecosystems health. For instance, only in Louisiana there are about 90,000 septic systems discharging to natural waters that drain to Lakes Pontchartrain and Maurepas (Fig. 3). Thus, EOCs, ARB and ARGs may be a significant problem in the Lake Pontchartrain Basin due to the large number of individual septic systems. Decentralized treatment systems where septic tanks are connected to CWs, natural wetlands or drainage fields offer an economical and effective way of reducing the concentrations of EOCs, ARB and ARGs from septic systems.

Decentralized treatment approaches for septic systems and package plants offer an economic way of achieving better treatment. In this approach, liquid waste is collected in a small diameter (2–3 in) flexible piping ditch-witched into the ground. This flow can then be further treated in a conventional STP, CW or a natural wetland. The solid waste

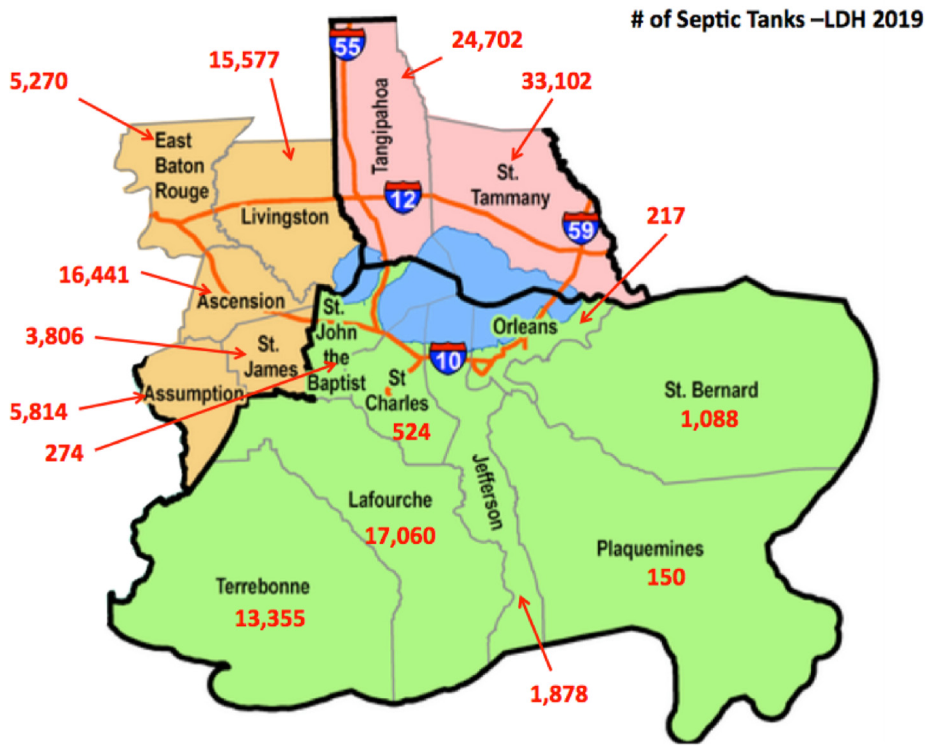

Fig. 3. Number of individual septic systems by Parish in southeast Louisiana. Lakes Pontchartrain and Maurepas are shown in the middle of the figure. (Source LDH 2019).

is pumped from the septic tank every few years and treated.

8. Reduction of PPCPs, ARB and ARGs in natural wetlands

Natural wetlands can also reduce PPCPs, as well as ARB and ARGs, in treated sewage effluents. Direct discharge to natural wetlands after treatment in a centralized STP can often result in significant removal of these compounds in a relatively small area. Hayward et al. (2018) reported that ARGs generally decreased in tundra wetland ecosystems receiving domestic wastewater, and that removal rates were inversely correlated with HRT. In this study, short HRTs (2 days) produced the highest ARGs absolute abundance concentrations in the effluents. The authors indicated that soils may act as a reservoir for ARB, potentially enriching overlying waters by desorption. Similarly to the study by Fang et al. (2017) in CWs, seasonal variability in the removal efficiency of the tundra wetlands was also observed, with lower removal rates during summer.

Conkle et al. (2008) measured the uptake of various pharmaceutically active compounds (PhACs) in the Mandeville, Louisiana wastewater treatment system (Table 3), which included aerated ponds, a constructed nitrification-denitrification wetland, and the Bayou Chinchuba natural forested wetland system that discharges to Lake Pontchartrain. Thirteen of 15 PhACs studied were detected in the wastewater inflow to the treatment plant. Nine of the 13 compounds were above detection limits in the effluent of the treatment plant and concentrations of most compounds were reduced > 90% within the plant, while carbamazepine and sotalol were only reduced by 51% and 82%, respectively. The removal rates observed in the Mandeville system including the forested wetland (Tables 3 and 4) were greater than those reported for conventional STPs, probably due to the longer total HRT (> 30 days). Most of the target PhACs were reduced to very low levels or below detection limits before discharge into the Lake Pontchartrain estuary, and their total annual loading was reduced from greater than 200 kg to less than 1 kg (Conkle et al., 2008).

Information from Conkle et al. (2008) can be used to estimate the area of wetland required to reduce PhACs to near background or non-detect levels. The dry weather discharge from the plant is about 6500 m^3/day. The area of wetlands required to achieve high reductions in PhACs is about 3800 m^3/day, considering the results by Conkle et al. (2008) in the overall treatment stream (STP + wetlands) would be about 23 ha. The area of the Bayou Chinchuba wetland is about 40 ha and the population of Mandeville is about 13,000 people and, thus, the per person equivalent (PE) to achieve the reduction rates reported by Conkle et al. (2008) is about 30 m^2. This equals to 2–8 m^2/PE for constructed wetlands and 0.06 m^2 for conventional STPs (Ávila and García, 2015) (Fig. 1). Additional reduction can be achieved with larger wetland areas. Because all assimilation wetlands in Louisiana have areas that are in excess of what is required to reduce nutrients to

Table 3
Percent removal of pharmaceutially active compounds from the wastewater treatment plant at Mandeville Louisiana (USA), from the receiving forested wetland and from the total System. Adapted from Conkle et al. (2008). ND = non-detected.

Class	Compound	STP (%)	Wetland Discharge	Total % Removal
Alkaloids	Cotinine	> 99	–	> 99
(estimulants)	Caffeine	> 99	–	> 99
Psychiatric drugs	Carbamazepine	−53	105	51
	Fluoxetine	ND	ND	ND
β-blockers	Atenolol	> 99	6	> 99
	Nadolol	77	23	> 99
	Propranolol	ND	ND	ND
	Metoprolol	92	8	> 99
	Sotalol	30	52	> 99
Antibiotics	Sulfapyridine	76	24	> 99
(sulfonamides)	Sulfamethoxazole	100	–	> 99
Anti-inflammatories	Acetaminophen	100	–	> 99
	Naproxen	99	1	> 99
	Ibuprofen	> 99	–	> 99
Lipid regulator	Gemfibrozil	64	31	95

Table 4
Percent removal and concentrations in treated wastewater for target pharmaceutical compounds in conventional wastewater treatment systems compared to constructed/natural wetland systems. Adapted from Conkle et al. (2008). CBZ = carbamazepine, SMX = sulfamethoxazole.

Compound	% Reduction in Wetlands	% Reduction in STPs	STPs effluent concentration (μg/L)
Caffeine	> 99	94–99	0.18–0.22
Carbamazepine	51	7–30	1.18–2.10
Gemfibrozil	91	69–75	0.18–0.40
Ibuprofen	99	90–96	0.15–0.37
Metoprolol	> 99	30–65	0.19
Naproxen	99	66–93	0.25–0.30
Sotalol	82	25	0.25
Sulfamethoxazole	92	24	0.62

background levels, it is likely that most if not all PPCPs are reduced to background or non-detectable levels in these more extensive systems.

Nutrient and sediment reduction in secondarily treated municipal effluents using natural wetlands (wetland assimilation) has a long history in coastal Louisiana (Hunter et al., 2018; Day et al., 2018). There are 11 assimilation wetlands, five of which have operated for 27–70 years, with more than a combined 250 system-years of operation. There has been extensive studies of the hydrology, biogeochemistry, vegetation productivity, decomposition rates, faunal communities and PPCPs dynamics in these assimilation wetlands in coastal Louisiana. These studies have demonstrated that, generally, nutrients are reduced to background levels before flowing into open water bodies, vegetation productivity and accretion are enhanced, and decomposition rates are not increased compared to reference wetlands not receiving input of treated effluent. The information included in this review suggests that an optimally managed assimilation wetland could serve to reduce PPCPs, ARB and ARGs.

The reduction of PPCPs in constructed and natural wetlands is facilitated by multiple removal mechanisms such as microbial degradation, sorption to soil particles, precipitation, plant uptake, photolysis, and hydrolysis (White et al., 2006). Highl organic soils in wetlands provide a sorption medium for a wide variety of variety of contaminants. The high microbial biomass and diversity in wetlands can promote the uptake and degradation of a wide variety of PPCPs including antibiotics. The existence of multiple aerobic/anaerobic interfaces in the wetland soil and in the oxidized plant rhizosphere enhances the degradation of organic compounds. In addition, the presence of rooted plants provides a substrate for the development of biofilms. The high soil surface area in relation to shallow water depth, compared to open water systems, allows PPCPs to be in contact with organic soil, microbial populations and biofilms associated with plants.

9. Conclusions

The presence of antibiotics selectively favors ARB and ARGs, so their reduction in wetlands needs to be an area of focus. Research on disinfection methods and the behavior of EOCs, ARB, and ARGs should continue to inform adaptive management of treatment systems to reduce the impact on the receiving environment, ensuring safety for both humans and other organisms. Overall, a well-managed treatment system coupling municipal wastewater treatment systems to wetlands can result in significant reductions of EOCs as well as ARB and ARGs into the receiving waters.

CRediT authorship contribution statement

Joan Garcia: Writing - original draft. **María Jesús Garcia-Galan:** Writing - original draft. **John W. Day**: Writing - original draft. **Raj Boopathy**: Conceptualization, Writing - review & editing. **John R. White:** Writing - original draft. **Scott Wallace:** Writing - original draft. **Rachael G. Hunter:** Writing - original draft.

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