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Letter

# Plant Uptake of Trace Organic Contaminants in Effluent-Dominated Streams: An Overlooked Terrestrial Exposure Pathway

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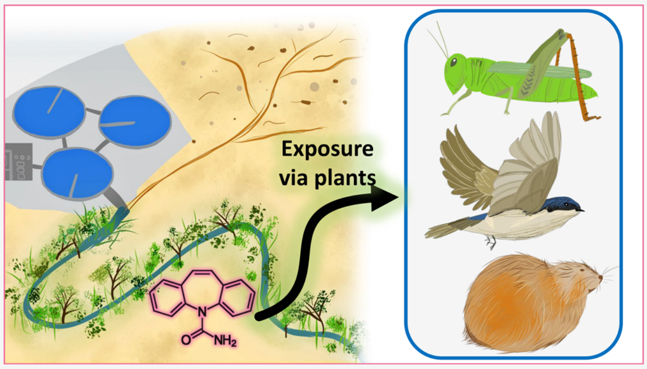
**ABSTRACT:** Uptake and translocation of water-soluble organic compounds by plants represent important means through which terrestrial organisms can be exposed to organic contaminants. In terrestrial systems adjacent to wastewater effluent-dominated streams (e.g., riparian corridors), insects, mammals, and birds could be exposed to trace organic contaminants through this pathway, especially in arid regions where other water sources are scarce. Analysis of the antiepileptic drug, carbamazepine, in plants grown under conditions that mimic effluent-dominated riparian zones indicates that concentrations in plant tissues exceed levels that may pose risks to herbivorous and/or detritivorous organisms. Plant uptake of carbamazepine exhibited considerable interspecies variability and distinct spatial and temporal trends. It also showed evidence of in-planta transformation. Because effluent-dominated riparian zones serve as important habitats for terrestrial species in arid regions, plant uptake and translocation of wastewater-derived trace organic contaminants should be considered when assessing the potential ecotoxicological impacts of municipal wastewater effluent discharges.




**KEYWORDS:** *Micropollutants, Ecotoxicity, Bioaccumulation, Wetland, Hydrophyte*

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## ■ INTRODUCTION

In arid and semiarid regions, municipal wastewater effluent is frequently the dominant source of water for rivers and streams downstream of cities. For example, municipal wastewater effluent accounts for more than 10% of the median flow in streams that serve as sources for drinking water treatment plants in the southwestern United States under typical flow conditions.[1] Across the entire United States, municipal wastewater effluent makes up more than half of the flow in over 900 streams. During dry periods, nearly all of the flow in some of these streams is derived from treated wastewater.[1−4] Over the past two decades, considerable research has been conducted on the effects of wastewater-derived trace organic contaminants on aquatic organisms. Results suggest that continuous exposure to wastewater effluent can lead to endocrine disruption, oxidative stress, behavioral alterations, and genotoxicity.[5−8] Despite the progress on understanding and managing these impacts, little attention has been directed toward the potential effects of wastewater-derived trace organic contaminants on terrestrial organisms.[9−11] Recently, pharmaceuticals and other trace organic contaminants were detected in emergent insects that developed in effluent-dominated streams, which highlights the potential for contaminant transfer from effluent-dominated water bodies to terrestrial ecosystems.[12−15]

Trace organic contaminants can undergo uptake and translocation when plants rely on municipal wastewater effluent as their main water source. For example, uptake of trace organic contaminants by plants has been well documented in agricultural systems employing treated wastewater for irrigation.[16−23] Results from multiple studies indicate that uncharged compounds of intermediate hydrophobicity (i.e., log $K_{ow}$ ranging from 2 to 4) are most readily taken up and translocated via the xylem. With the exception of relatively volatile compounds (e.g., trichloroethylene),[24] translocated trace organic contaminants and/or their metabolites tend to accumulate in leaves, stems, fruits, seeds, and pollen.[25−27]

In riparian zones, herbivory and detritivory result in transfer of nutrients and, potentially, contaminants of anthropogenic origin from aquatic to terrestrial systems.[28,29] In arid environments, riparian vegetation is particularly important to invertebrates and wildlife because it is a major source of food and/or water.[30] Considering this context, we hypothesized that

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uptake of trace organic contaminants by plants could create an important contaminant exposure pathway for terrestrial organisms in effluent-dominated riparian zones and wetlands. To test this hypothesis, we characterized plant uptake and in-planta transformation of carbamazepine in a constructed subsurface wetland system that mimics the conditions encountered in riparian zones and other wetlands where water primarily flows through the subsurface. We selected carbamazepine as the target analyte because it is persistent under the typical conditions found in effluent-dominated streams.[31,32] Also, it is frequently detected in rivers globally (e.g., in 62% of global rivers in a recent survey[33]) at concentrations that may pose ecotoxicological risks to aquatic life,[34] and its uptake, in-planta metabolism, sequestration into different parts of plants and interspecies variability has been well characterized in agricultural systems and in some hydrophytic plants.[16,35−41] Because plant litter is an important food source in aquatic and terrestrial foodwebs,[42] we evaluated the transformation of carbamazepine in decomposing plant litter.

## ■ MATERIALS AND METHODS

As described in detail previously,[43] the 0.7 ha field site consisted of 12 parallel subsurface wetland cells with two types of plant communities[43,44] (Figure S1). All cells were hydraulically isolated from each other and from groundwater. Nine of the 12 cells were planted with meadow vegetation (e.g., sedges and rushes). Three cells were planted with willows (i.e., *Salix lasiolepis*) and riparian vegetation. Three meadow cells and three riparian cells were included in this study (details about selecting the wetland cells for this study are found in Section S1.1 of the SI). Municipal wastewater effluent was continuously released at a depth of about 1 m below the surface near the entry to the cells starting in April of 2017.[43,45,46]

Live plant samples were collected between June 2019 and June 2021. Horizontal transects were installed at approximately 0, 7.5, 15, 22.5, 30, 37.5, and 45 m from the inlet perpendicular to the flow of water in three meadow wetland cells (Figure S1). Approximate spatial abundance and distribution of plant species were determined by identifying plants along each transect. Species with the greatest distribution and abundance (i.e., *Bolboschoenus maritimus*, *Juncus* spp., *Baccharis glutinosa*, and *Euthamia occidentalis)* were selected for collection. Along each transect, a spatially representative subset of these plants was collected as whole plant shoot samples in June 2019. *Juncus* spp. samples were collected using the same method again in June 2021. Individual willow trees (i.e., *S. lasiolepis)* were tagged in triplicate riparian cells at different distances along the flow path, and mature leaves were sampled from the same trees multiple times over the growing season (Figure S1). Samples were stored on ice prior to returning to the lab.

In November 2019, fresh *B. maritimus* and *Juncus* spp. samples were collected at the entrance of the meadow cells for litterbag experiments. After returning to the lab, *B. maritimus* and *Juncus* spp. were subsampled into preweighed 40 cm × 40 cm polypropylene mesh litterbags. Fresh litterbags were then returned to the site and placed on the sediment surface, under the plants that were growing on the surface. Periodically, litterbags were sacrificially sampled.

In the laboratory, all plant parts (e.g., leaves, whole plant shoots, or litterbag samples) were manually cut into 1−2 cm pieces, homogenized, and shredded with a Cuisinart DCG-12BC grinder. Homogenized plant samples were packed into 15 mL glass vials before freeze-drying. After freeze-drying was completed, samples were ground into a fine powder using a BioSpec Products mini-beadbeater and stored at −20 °C. Samples of freeze-dried and frozen plants were stored for no longer than two years. Details about the impacts of storage time on the quantification of carbamazepine can be found in Section S1.5 of the SI.

Solid−liquid extraction was performed using modified versions of published methods.[47] Briefly, 0.1 g of freeze-dried, powdered plant material was extracted in 4 mL of a 3:5 Milli-Q water:methanol solution via sonication and shaking. The supernatant was decanted and reserved. The process was repeated with 2.5 mL of a 1:1 Milli-Q:methanol solution. Combined supernatants were filtered through a 0.2 $\mu$m polyethersulfone membrane. An additional extraction step was performed to ensure that no residual organic contaminants could be detected, and the method was validated via spike-recovery tests (Section S1.3 of the SI).

Prior to analysis, extracts were diluted 1:2 in Milli-Q water and spiked with a mixture containing 5 ng of each internal standard compound (Table S5). Carbamazepine and carbamazepine metabolites were analyzed using liquid chromatography mass spectrometry on an Agilent 1260 HPLC coupled to an Agilent 6460 triple quadrupole mass spectrometer. A matrix-matched calibration curve was prepared using extracts from unexposed *S. lasiolepis* leaves collected from mature trees that received rainwater and potable water on the UC Berkeley campus. Additional details about preliminary experiments performed in the winter of 2019, extraction methods, LC-MS/MS methods, and the methods used to estimate dietary exposures of wildlife can be found in Section S1 of the SI.

## ■ RESULTS AND DISCUSSION

Carbamazepine and its main plant metabolites were detected and quantified in 81% and 62% of the plant residue samples, respectively (Figure 1, Figures S4−S6). Concentrations of carbamazepine in this study were in the same order of magnitude as levels found in field-grown agricultural plants that were irrigated with treated wastewater.[22] In addition, concentrations of carbamazepine in the leaves and stems of most live meadow plants decreased along the path of water flow; concentrations of carbamazepine in plants were inversely correlated with distance from the inlet in the meadow cells ($p < 0.05$, Spearman's rank) (Figure 1). For *Juncus* spp., *B. maritimus*, and *E. occidentalis*, median carbamazepine concentrations decreased by approximately 60% between the inlet and outlet of the wetland. *S. lasiolepis* leaves collected at 7.5 m from the inlet contained the highest concentrations of carbamazepine among the riparian cells. The spatial trend of carbamazepine in meadow plants was consistent with concentrations measured in porewater samples taken along the path of water flow; concentrations decreased by about 50% within the first 15 m of the wetland.[48] The mechanisms of carbamazepine removal in the wetland will be described in future publications.[48]

Concentrations of carbamazepine exhibited considerable variability among plant species. At the start of the wetland, the median carbamazepine concentrations in *Juncus* spp. and *B. maritimus* were approximately twice those of *B. glutinosa* and *E. occidentalis* (Figure 1). A similar or even greater degree of interspecies variability has been observed in agricultural plants (e.g., concentrations of carbamazepine in lettuce were


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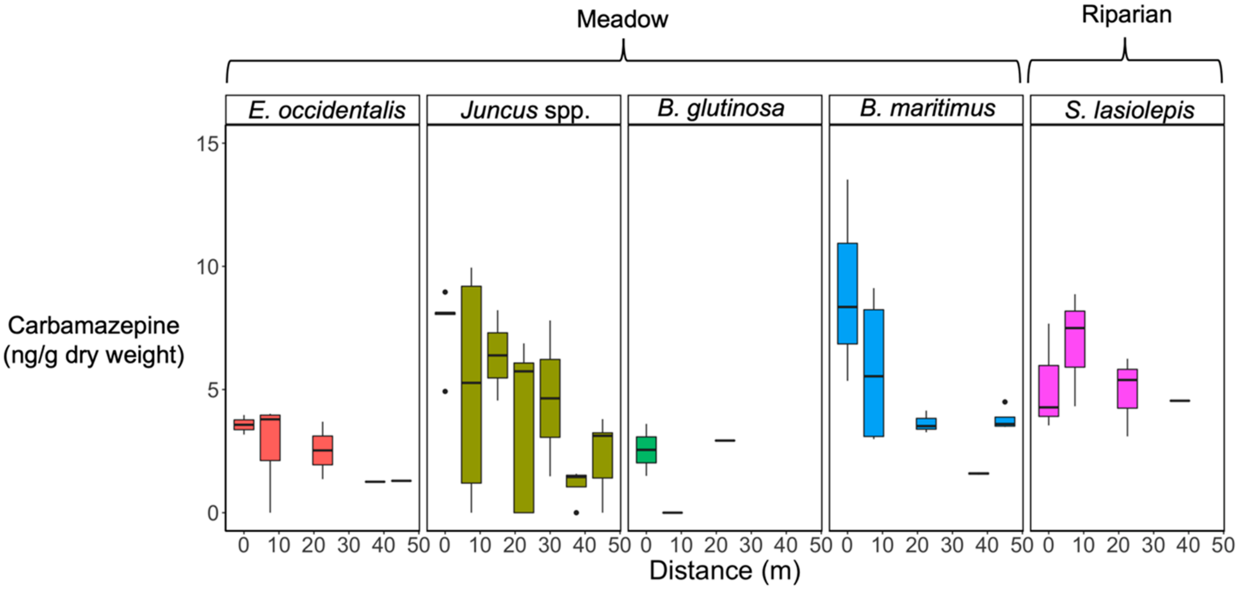


**Figure 1.** Concentrations of carbamazepine in wet meadow and riparian plant species grown under field conditions. *S. lasiolepis* leaves were collected in December of 2019. Meadow plants were collected in June of 2019 and 2021. In boxplots, $n$ = 3−5. Horizontal lines represent locations where $n$ = 1. $n$ is only less than 3 in cases where plant species were not located at all sampling distances.

approximately six times those found in spinach).[25] Interspecies variability was likely due to differences in root lipid content, development of the Casparian strip (i.e., a barrier preventing free water and ion flow from plant roots into above ground tissues via the xylem), evapotranspiration rates, expression of carbamazepine-metabolizing enzymes, or other physiological factors.[39]

Carbamazepine gradually accumulated in *S. lasiolepis* leaves during the growing season. Mean carbamazepine concentrations in *S. lasiolepis* leaves from the same tree more than tripled between June and December of 2019 (Figure 2; Figure S8). Although temporal variations were not measured in other perennial plants, we anticipate that concentrations would also increase in those plants during the growing season because plants would continue to transpire carbamazepine-laden water throughout the entire season. Therefore, concentrations of carbamazepine in meadow plants may have been higher in September and October than at the time the samples were collected (i.e., June). Future efforts to quantify wildlife exposures at a population level should account for temporal variability because wildlife habitat use within home ranges (e.g., herbivorous mammalian breeding[49,50]) differs across seasons.

Metabolites of carbamazepine were detected in all plant species (i.e., metabolites were detected in 12%−42% of the plant samples), but these metabolites could not be quantified using the matrix-matched methods employed in this study due to higher limits of quantification for those compounds relative to carbamazepine (Figures S5 and S6; Table S6). However, we estimate that detectable metabolites were present at levels that were of a similar order of magnitude as carbamazepine, which is consistent with data from agricultural plants.[37] The presence of metabolites of carbamazepine in plant tissues suggests that carbamazepine is transformed in plant tissues under field conditions because some of these metabolites (e.g., 10-hydroxy carbamazepine) were not observed in porewater more than 5 m from the inlet but were present in plant samples collected throughout the wetland (Figures S5 and S6). Also, some of the metabolites that were detected in the wetland influent and porewater (e.g., dihydroxy-carbamazepine) may not be as readily taken up by plants due to their higher hydrophilicity (log $K_{ow}$ < 1).[35]

Concentrations of carbamazepine gradually decreased in dead *Juncus* spp. and *B. maritimus* exposed to field conditions in litterbags likely due to leaching from plant material or microbial transformation reactions (left panel of Figure 2; Figure S9). In most decomposing plant samples, carbamazepine metabolites were below the limits of quantification, limiting our ability to attribute carbamazepine removal to transformation. However, a suite of carbamazepine metabolites was detected in senescing plants collected during preliminary experiments conducted in the winter of 2019 (Figure S10). Regardless, the loss of carbamazepine from decomposing plants was slow (i.e., the carbamazepine half-life was greater than one and a half months) and suggests that that the compound may linger for months in detritus layers (Figure 2). Due to seasonal growth cycles, concentrations in detritus would likely be highest during winter months after plant senescence occurs or deciduous trees drop their leaves.

We presume that plant uptake and translocation exposes detritivorous or herbivorous insects, birds, and mammals to carbamazepine because research on human exposure to carbamazepine via wastewater-irrigated food crops indicates that the compound is bioavailable when plant tissues are ingested.[51] Using published estimates of daily dietary intake,[52] we predict that, during the summer, herbivorous birds and mammals (e.g., Canada goose and muskrat) feeding exclusively on plants grown in a 0−7.5 m strip adjacent to the water source would ingest approximately 0.4 to 2.4 $\mu$g kg-body weight$^{-1}$ d$^{-1}$ of carbamazepine. This estimate represents a worst-case scenario because it assumes that birds and mammals consume diets comprised exclusively of vegetation with the highest concentrations of carbamazepine (i.e., plants growing near the inlet). Additional research is needed to assess feeding patterns of different bird and mammal species under these conditions. Regardless, herbivorous insects can consume up to


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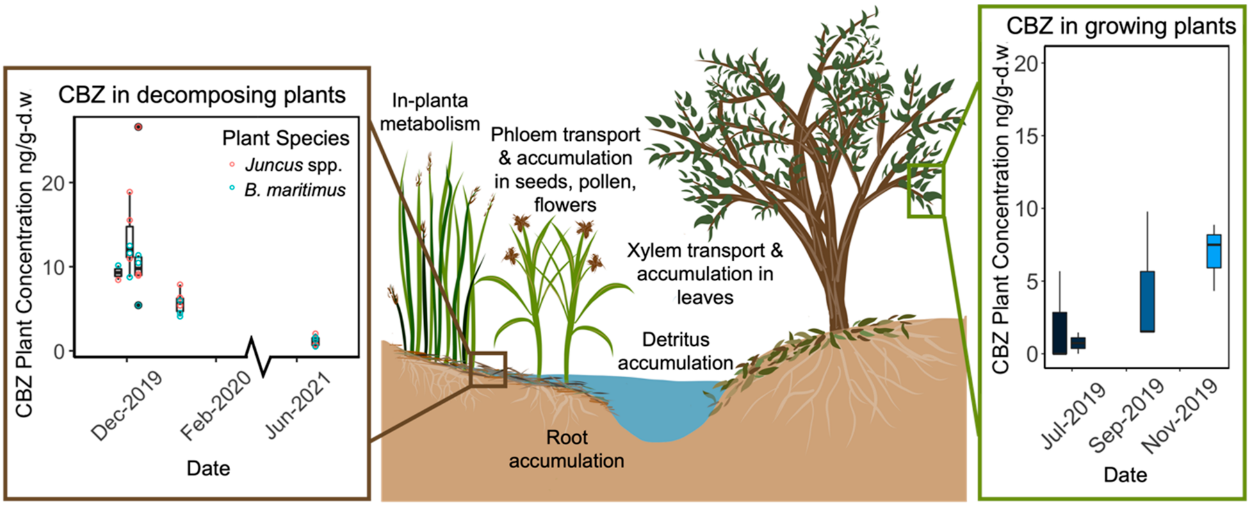


**Figure 2.** Visual rendering of a cross-section of effluent-dominated riparian zones and wetlands. Major in-plant transport and transformation processes are labeled in the center. The left plot displays concentrations of carbamazepine over time in decomposing *Juncus* spp. and *B. maritimus* in litterbag experiments. The box and whisker plots summarize the full data set (represented by colored, unfilled points); outliers are plotted as black dots with the colored unfilled data point overlaid on top. The right plot shows concentrations of carbamazepine in *S. lasiolepis* leaves over the growing season. Leaf samples were collected from individual trees located 7.5 m from the inlet. Data from samples collected at other distances are found in Figure S8.

one and a half times their body weight of plant matter per day, resulting in a weight-normalized dietary exposure that is higher than that received by birds and mammals.[53] Thus, predatory insects, birds, and mammals feeding on herbivorous insects might also be exposed to the compound, as has been previously reported for insectivorous birds and invertebrates feeding on aquatic emergent insects.[13,54]

Unfortunately, little is known about the impact of pharmaceuticals on terrestrial herbivorous wildlife and invertebrates.[11] A few studies have considered the potential effects of wastewater-derived trace organic contaminants on herbivorous invertebrates (e.g., agricultural pests); results indicate adverse impacts when *Trichoplusia ni* are reared on artificial diets or hydroponically grown plants containing environmentally relevant concentrations of trace organic contaminants.[55,56] However, these studies did not consider the effects of carbamazepine specifically. Therefore, to contextualize our results, we compared our measurements to available data on the toxicity of carbamazepine. For example, the insect *Nauphoeta cinerea* (i.e., the speckled cockroach) exhibited behavioral shifts and oxidative stress when exposed to food containing carbamazepine at concentrations that were 39%−88% of the levels observed in the plants collected near the inlet to the wetland in this study.[57] Also, the concentrations measured in plants collected near the inlet of the wetland in our study were approximately 4%−11% of the $EC_{10}$ that blocked pupation and emergence in the nonbiting midge, *Chironomus riparius,* in chronic sediment-exposure experiments. Lastly, the bird and mammal exposures we discussed above were one to seven times the acceptable daily intake (ADI) for humans (0.34 g kg-body weight$^{-1}$ d$^{-1}$, Figure S11). These comparisons suggest that carbamazepine in plants may pose risks to terrestrial wildlife and invertebrates, but more data on relevant end points and species are needed for accurate risk assessments. In addition, there are significant limitations in comparing wildlife exposures to the human ADI because it employs cancer as an end point, incorporates various safety factors, and is not likely to be directly transferrable across species with differing physiologies.

Other aspects of terrestrial exposure pathways further complicate matters. Some organisms may receive higher or lower doses of carbamazepine through exposures to different parts of the plant (e.g., flowers, seeds, nectar, pollen, xylem sap, phloem sap) because wildlife and insects selectively feed on specific plant organs, and transport of carbamazepine into different plant compartments varies widely. For example, many birds and some mammals feed primarily on the seeds of *Juncus* spp. and *B. maritimus* as opposed to their live stems and leaves.[58] Carbamazepine has been shown to contaminate pollen via root uptake in zucchini irrigated with treated wastewater amended with carbamazepine.[27] To provide insight into these exposure pathways, future efforts should include measurements of carbamazepine and other wastewater-derived contaminants in various wetland and riparian plant compartments.

Metabolism is also an important factor to consider because carbamazepine is rapidly metabolized by the CYP 450 family of enzymes to produce hydrophilic products. CYP 450 enzymes are prevalent in many terrestrial plants, invertebrates, and vertebrates, but they may be less effective at low concentrations because carbamazepine metabolism is dose-dependent in humans.[59] The main metabolite produced by CYP 450 enzymes, carbamazepine epoxide, is toxic and may be teratogenic in humans.[60] Similar metabolites may form if carbamazepine is transformed by enzymes present during decomposition (e.g., lignin-degrading enzymes).[61] Therefore, both herbivores and detritovores could be exposed to carbamazepine and its metabolites in plants.

Although carbamazepine was the focus of this study, other trace organic contaminants can be taken up into plants and translocated to above-ground tissues (Figure S7).[18,23,62,63] For example, primidone and phenytoin—two relatively stable compounds—have been detected in cabbage and lettuce irrigated with treated wastewater.[25] Likewise, plant uptake and translocation could be an important entry pathway for short-


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chain per and polyfluoroalkyl substances that are persistent and bioaccumulative in terrestrial systems.[64−69] Finally, potent systemic insecticides such as fipronil, which can be present in treated wastewater at concentrations as high as 340 ng/L,[70] could undergo plant uptake and translocation because these insecticides are designed to be distributed throughout plants after application to seeds.[71] Attempts to quantify fipronil in the plants from this study were hindered by high background levels of the compound detected in the willow leaves used for matrix matching, low porewater concentrations, and relatively high limits of quantification for the compound. The willow leaves that were used for matrix matching could have had elevated concentrations of fipronil because they were collected from a site that is exposed to urban runoff. However, when compared to the willow leaves used for matrix matching, fipronil was detected at higher concentrations in 37% of the plant samples collected from the wetland cells. Regardless, fipronil and several of its primary metabolites have been detected in the roots and shoots of emergent hydrophytes collected from a surface flow wetland treating water from a wastewater effluent-dominated river in southern California.[72] The potential for terrestrial plants to be contaminated with fipronil is concerning because it is extremely toxic to invertebrates. In addition, plants growing in the presence of wastewater effluent would likely contain multiple trace organic contaminants which may pose additional risks due to mixture effects.

Many diverse plant species grow in effluent-dominated riparian zones and wetlands. As indicated by our data and data on crop uptake of contaminants, different species exhibit varying tendencies to take up carbamazepine and other trace organic contaminants.[25] Different plant species also are important to the diets of herbivores. For example, most insects are highly specialized and will only feed on a single plant species.[73] Thus, efforts to predict exposure to organisms in riparian zones would benefit from the application of species-specific data.

Because wastewater effluent accounts for an increasing fraction of the flow in the streams and rivers of water-stressed regions,[74] the importance of plant uptake as an exposure pathway for terrestrial organisms could increase in the future. In addition, riparian zones, wetlands, and other ecotones at the aquatic−terrestrial interface are more important than implied by their land area alone.[75] For example, 80% of terrestrial vertebrate species rely on riparian zones for food, water, and shelter in the American west, even though they account for only 5% of the land area.[76] Riparian zones also support a majority of neotropical migratory birds and provide critical habitat corridors for the migration and dispersal of land-bound wildlife.[77] Although wastewater effluent can create or restore riparian habitats that otherwise would not exist,[4] these habitats could also unintentionally expose organisms to contaminants. Considering this context, there is a need to document plant uptake of trace organic contaminants and its impacts on terrestrial wildlife and insects living and feeding in wastewater-impacted riparian zones and wetlands. These efforts will require environmental engineers and chemists to work collaboratively with ecologists and ecotoxicologists to ensure relevant ecotoxicity end points, receptors, and exposure pathways are holistically assessed in these important ecosystems.[78]

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.2c00543.

Maps of the field site, sampling procedures, analytical methods, exposure estimate calculations, details about data quality including extraction recoveries, effects of sample storage, and additional visualizations of results (PDF)

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### Notes

The authors declare no competing financial interest.

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