Effects of tetracycline, sulfonamide, fluoroquinolone, and lincosamide load in pig slurry on lettuce: Agricultural and human health implications

V. Matamoros a,*, M. Escolà Casas a, E. Pastor a, D. Tadić a, N. Cañameras b, N. Carazo b, J.M. Bayona a

a Department of Environmental Chemistry, IDAEA-CSIC, C/Jordi Girona, 18-26, E-08034, Barcelona, Spain

b Department of Agri-Food Engineering and Biotechnology DEAB-UPC, Esteve Terrades 8, Building 4, Castelldefels, Spain

1. Introduction

Intensive livestock production results in large amounts of animal dejections, which can contaminate water bodies with excess nutrients if they are not properly managed. In the EU-27 and the UK, it is estimated that 1.4 billion tons of livestock manure were produced annually between 2016 and 2019 (Königer et al., 2021). Manure is used in agriculture as a soil additive, either treated or untreated, to at least partially replace mineral fertilizer. However, the load of antimicrobial agents such as veterinary antibiotics (ABs) in manure also raises concerns regarding the environmental, agricultural, and human health implications of the spread of these compounds in agroecosystems (Xie et al., 2018; You et al., 2020). Several studies have shown that these compounds induce co- or cross-resistance to ABs in bacteria (Murray et al., 2019). According to WHO estimates, antimicrobial resistance (AMR) is among the biggest threats to global human health and food security; it is currently estimated to be responsible for 700,000 human deaths per year worldwide, a figure that will swell to 10 million if urgent action is not taken (WHO, 2019).

The concentration of ABs in manure ranges from undetectable to 1420 mg/kg in fresh weight (fw) depending on the farm management (Wohde et al., 2016). Crop uptake of sulfonamides, fluoroquinolones, lincosamides, and tetracyclines through manure fertilization has been reported in different settings (Barrios et al., 2020; Dolliver et al., 2007; Kang et al., 2013; Kumar et al., 2005a; Margenat et al., 2020; Mullen et al., 2019; Shen et al., 2021). Recent studies have also identified several transformation products (TPs) from ABs in crops (Dudley et al., 2018; Huynh and Reinhold, 2019; Tadić et al., 2021; Tian et al., 2019), but there is no information on their role in AMR selection.

As for the agricultural implications, at low concentrations, human and veterinary antibiotics induce hormesis in plants (Agathokleous et al., 2018; Migliore et al., 2010), but phytotoxic effects have been reported at higher concentrations (Jin et al., 2009; Rocha et al., 2021). Current laboratory-scale studies indicate that the presence of ABs in irrigation water may induce a morpho-physiological response in Populus alba (Pierattini et al., 2016) due to their potential to change plant...
phenotypic (e.g., biomass production, shoot and root growth) and metabolic (e.g., nitrogen metabolism, oxidative metabolism, and photosynthesis) profiles (Jin et al., 2009; Liu et al., 2009; Rocha et al., 2021). Previous greenhouse studies suggest that the pig-slurry fertilization dose does not affect lettuce uptake of ABs (Margenat et al., 2020), although increases in the AB concentration in pig slurry do increase their concentration in alfalfa crops (Huang et al., 2021; Kumar et al., 2005b). In any case, the novelty of this study stands on the fact that there is currently no available information regarding the agronomic and human health effects of the AB dose in manure on horticultural crops such as lettuce.

This study thus aims to explore plant uptake of ABs and the agronomic and human health impact thereof by conducting a dose-response assay under greenhouse conditions using pig slurry spiked with ABs (lincomycin, sulfadiazine, oxytetracycline, and enrofloxacin) at realistic environmental concentrations (0, 0.05, 0.5, 5, 50, 500 mg/kg fw). ABs have been selected to include at least one compound from each of the most frequently detected AB classes in pig-slurry (lincomycin, sulphonamides, fluoroquinolones, and tetracyclines). These ABs and their concentration levels were selected based on their abundance in pig slurry (Wohde et al., 2016) and the fact that they have been detected in crops following soil fertilization with manure (Matamoros et al., 2022; Tasho and Cho, 2016).

2. Material and methods

2.1. Experimental design

The experiment was conducted in a glass greenhouse facility located at the Agrópolis-UPC agricultural experiment station in Viladecans (Barcelona, Spain). Lactuca sativa L. cv. Maravilla de Verano was selected as the horticultural vegetable, and a total of 30 units were placed individually in 2.5 L amber glass pots (15 cm diameter, 20 cm high) with an inverted bottle shape fitted with a bottom outlet connected to drainage tubing (0.5 cm diameter) and filled with 2.3 kg of soil sieved through a 2 mm sieve (Hurtado et al., 2017) (Fig. 1 and Fig. 1-SM). The soil had a loamy clay texture (43.9% sand, 27.4% silt, 28.7% clay), a pH of 9, and an electrical conductivity of 0.19 dS/m. ABs and their concentration levels were set according to the amount of pig slurry used in the experiment (Wohde et al., 2016) and the fact that they have been detected in crops following soil fertilization with manure (Matamoros et al., 2022; Tasho and Cho, 2016).

2.2. Sampling strategy

Lettuces were assessed for general agricultural quality parameters and homogenized for antibiotic analysis. For metabolomics, the five lettuces from each treatment were analyzed. To this end, 5 middle- and old-stage leaves from each selected lettuce were collected using an 8-mm leaf punch disk and were immediately frozen on dry ice. The samples were then stored at −80 °C until analysis. Immediately after the metabolic sampling, the lettuces were harvested, weighed, and measured for leaf height and number of leaves.

2.3. Antibiotic and transformation product analysis

The determination of ABs in the pig slurry and vegetables was performed using a previously described methodology (Berendsen et al., 2015). Briefly, 0.5 g fw of samples was extracted by ultrasound-assisted extraction with McIlvaine buffer with ethylenediaminetetraacetic acid (EDTA) (pH = 4). This was followed by protein precipitation, centrifugation, and solid-phase extraction (Strata X) cleanup. The final determination was performed by LC-ESI-MS/MS in the multiple-reaction mode with two transitions per compound. For the determination of ABs and TPs, two different procedures were used: one for tetracyclines and another for all other ABs. The procedure for ABs other than tetracyclines was based on a method described elsewhere (Tadic et al., 2019); in the procedure for tetracyclines, the McIlvaine buffer (pH = 4)-EDTA solution was replaced with aqueous McIlvaine buffer (pH = 4)-EDTA solution. Parent antibiotics were determined by ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS) (Waters TQD).

AB TPs were analyzed with a Thermo Q Exactive Orbitrap. Extracts were analyzed with an HPLC Accela 600 pump coupled to a Q-Orbitrap HRMS mass spectrometer (Thermo Fisher Scientific) equipped with a heated electrospray ionization probe (HESI) source for detection. Chromatographic separation was done using a Waters XBridge BEH C18 column (2.1 × 150 mm, 2.5 μm particle size) equipped with a precolumn. The chromatography assays involved a 10 μL injection volume, a 0.30 mL/min flow rate, and a binary gradient of water (A) and acetonitrile (B), both containing 0.1% formic acid, as follows: 10% B at 0–1 min, 90% B at 10–23 min, 10% B at 24–29 min. The HESI parameters were as follows: 55 arbitrary units (AU) sheath gas; 10 AU auxiliary gas; 275 °C capillary temperature; 200 °C heater temperature; and an
Electrospray voltage set at 4.0/−4 kV. The S-lens radio frequency (RF) level was set at 100 A.U. Full scan data were acquired at a resolution of 70,000 full width at half maximum (FWHM) with an automatic gain control (AGC) of $10^5$, 250 ms of the maximum ion injection time, and a scan range of 100–800 m/z. For MS², data-dependent acquisition (DDA) and data-independent acquisition (DIA) were achieved at a resolution of 17,500 with two absolute collision energies (20 eV and 40 eV) using an isolation window of 1 m/z, an AGC of $5 \times 10^4$, 150 ms of the maximum ion injection time, and a scan range 50–800 m/z. Lettuce samples grown with the highest concentration of ABs (Dose 4) were selected for the analysis of AB TPs, and samples grown without fortification were used as control samples. The investigation of AB TPs was performed in two steps. In the first step, DIA and in-depth analysis of in-silico-predicted TPs of the four analyzed ABs provided a list of TP candidates. This list was used as the inclusion list for the second step, which provided the final confirmation of TPs based on their fragmentation pattern, which was obtained using DDA. The in-silico transformation prediction, data mining, and data processing were done using Compound Discoverer 3.2 and FreeStyle 1.7 software (Thermo Fisher Scientific).

2.4. Agronomic parameters

The dry and fresh biomass and the length and number of lettuce leaves were measured at the end of the productive cycle. The chlorophyll content in leaves and biomass weight were measured in situ. Chlorophyll was gauged using a chlorophyll-meter (Opti-Sciences, Hudson, NH, USA). Lipid and carbohydrate content were measured as described elsewhere (Margenat et al., 2018).

2.5. Plant metabolomics

The frozen punch disks were homogenized with a TissueLyser LT (Qiagen) sample disruptor. Next, 10 mg of homogenized lettuce material was transferred to an Eppendorf tube, and 400 μL of methanol was added. The samples were then vortexed, sonicated, and centrifuged with methanol, chloroform, and water following a previously described methodology (Hurtado et al., 2017). The extracts were vacuum-dried and derivatized with methoxamine in pyridine (20 mg/mL) and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) according to Jorge et al. (2016). At this stage, 25 μL of sample aliquots was taken, mixed, and used to generate five quality controls (QCs). Finally, 17 μL of triphenylamine (at 4.5 ppm) was added to the samples and QCs. 2 μL of sample was injected in a Q Exactive gas chromatography (GC)-Orbitrap with a Zebron ZB-5HT In-Ferno column (30 m × 0.25 mm 0.25 μm). For the run, oven temperature started at 70 °C for 2 min, before being increased to 100 °C at a speed of 7 °C/min. The temperature was then increased again to 260 °C, at 5 °C/min, and finally to 320 °C, at 10 °C/min, for 5 min.

Each GC-Orbitrap datadfile was deconvoluted to extract individual peaks from the total ion chromatogram (TIC) using the Deconvolution Plugin application that was installed on the standard data-processing software from Thermo Scientific (TraceFinder 5.1 EFS software). To obtain the peaks for identification, a signal-to-noise ratio cut-off of 50, a mass tolerance of 5 ppm, a minimum TIC intensity of 1 × 105 and an ion overlap window of 98% were used. All ions in the deconvoluted spectra from the peaks were used for the subsequent library search (in NIST). As high-resolution data was used, only tentative hits with a high-resolution filtering (HRF) > 90 were selected. The use of HRF allowed us to select only features whose spectrum could be explained by more than 90% of the chemical formula proposed from the best library match result.

2.6. Human health risk assessment

The hazard quotient (HQ) was calculated using the estimated daily intake (EDI, μg/kg/day) and the acceptable dose intake (ADI) approach, as described elsewhere (Margenat et al., 2019). The HQ is the ratio

Table 1
Physicochemical properties of the spiked antibiotics in pig slurry.

<table>
<thead>
<tr>
<th>Compound (CAS) Structure</th>
<th>MW</th>
<th>Solubility* (mg/L)</th>
<th>pKa</th>
<th>Log Kow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetracycline (79-57-2)</td>
<td>460</td>
<td>313</td>
<td>3.57</td>
<td>−0.9</td>
</tr>
<tr>
<td>Enrofloxacin (93,106-60-6)</td>
<td>359</td>
<td>&gt;53</td>
<td>6.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Sulfadiazine (22,199-08-02)</td>
<td>250</td>
<td>77</td>
<td>6.5</td>
<td>−0.09</td>
</tr>
<tr>
<td>Lincomycin (154-21-2)</td>
<td>406</td>
<td>927</td>
<td>7.6</td>
<td>0.56</td>
</tr>
</tbody>
</table>

* Measured at 25 °C.

Fig. 2. Concentration of ABs in lettuce leaves along with the AB dosage in the pig slurry. D5 is not shown as plants did not survive. The following concentrations were spiked in the pig slurry: D0 = 0; D1 = 0.05 mg/kg fw; D2 = 0.5 mg/kg fw; D3 = 5 mg/kg fw; D4 = 50 mg/kg fw, and D5 = 500 mg/kg fw.

between the EDI and threshold levels considered to be acceptable daily intakes (ADIs).

For the calculation of the EDI, the 95th percentile was taken as a reference value. The ADI (μg/kg/day) values used were taken from a list based on microbiological and toxicological endpoints compiled by Wang et al. (2017) (Table 1-SM). The THQ is estimated as the sum of the individual HQs. If the THQ is less than 1, the risk is generally deemed acceptable.

2.7. Data analysis

Differences between agronomical parameters or AB content in vegetables were determined by the Kruskal-Wallis test using IBM SPSS v25 and R software. The 0.05 level of significance was used. For data analysis of metabolomics data, a matrix containing the feature peak areas from each sample and the QCs was uploaded to the MetaboAnalyst 5.0 server (http://www.metaboanalyst.ca/) (Pang et al., 2021). The data quality was checked, and features showing more than 20% variance among QCs were removed from further analysis. Subsequently, the data were normalized by the sum of ions and scaled with autoscaling
observed by Sallach et al. (2018), who studied the uptake of three antibiotics (sulfamethoxazole, lincomycin, and oxytetracycline) by lettuce plants grown in three soils with variability in texture (loam, sandy loam, tilled) under controlled conditions. In the present research, the hydroxy/sulfate-derivatives were identified in the lettuce translocation factor of this compound (Shen et al., 2021). We speculate that the reason why oxytetracycline was not detected in lettuce leaves could be due to the fact that it behaves as a zwitterion at the pH conditions, oxytetracycline was only detected in alfalfa leaves when spiked at 5 mg/kg fw. These results are in keeping with those reported by Bhardwaj et al. (2009), who found that veterinary ABs played an important underlying role in driving the negative effects on peanut grain yields by interfering with the available nutrient content in microbe- and earthworm-mediated soil. Recent studies suggest that ABs such as oxytetracycline have a negative effect on P uptake and shoot growth (Li et al., 2020; Zhang et al., 2021). In all cases, the polarity of the molecule would increase due to the additional oxygen in the molecular scaffold and lead to their elution earlier than the lincomycin, as it showed two main fragments – m/z 359.2176 and m/z 126.1276 – which are the two main characteristic fragments of lincomycin (https://www.mzcloud.org/DataViewer or mzCloud Database). Hence, it can be concluded that the detected compounds are two stereoisomers of hydroxyl lincomycin or hydroxyl lincomycin and lincomycin sulfoxide, as the mass difference between the TPs and the parent compound indicates additional oxygen (+15.9948). In both cases, the polarity of the molecule would increase due to the additional oxygen in the molecular scaffold and lead to their elution earlier than the lincomycin (RT = 4.71 min), as was observed. However, it was practically impossible to distinguish between these two possibilities due to the limitation of the LC-HRMS, to which end, NMR would be more suitable.

### 3.3. Agricultural implications

Table 2 shows that crop productivity, assessed as fresh weight, was only statistically compromised at D4, whereas at D5 (500 mg/kg fw) lettuce vegetables did not survive. This reduction in lettuce yield at an AB concentration level of 50 mg/kg fw can be explained by a phytotoxicity effect of the presence of ABs, as confirmed by the fact that when the AB concentration was increased to 500 mg/kg, the lettuce crops did not survive. These results are consistent with numerous studies that have reported the phytotoxicity of veterinary ABs on crop growth due to their ability to inhibit root activity, alter microbial community structure, enhance or overwhelm the enzyme system responses, and cause oxidative stress (Liu et al., 2009; Uddin et al., 2019). Zhao et al. (2022) observed that the increased prevalence of veterinary ABs in agro-ecosystems induced earthworm abundance and bacterial diversity, and thus decreased the bioavailability of soil nutrients. Analysis indicated that veterinary ABs played an important underlying role in driving the negative effects on peanut grain yields by interfering with the available nutrient content in microbe- and earthworm-mediated soil. Recent studies suggest that ABs such as oxytetracycline have a negative effect on P uptake and shoot growth (Li et al., 2020; Zhang et al., 2021). Chlorophyll content was not affected by any AB dose, but carbohydrate content decreased in lettuce exposed to a very low concentration level of ABs in the pig slurry (25 mg/kg). These results are in keeping with those reported by Bharathraj et al. (2009), who found that soluble carbohydrate content in Phaseolus vulgaris L. plants decreased with increasing concentrations of heavy metals (Pb and Cd). Similarly, Galal et al. (2021) found that proteins and carbohydrates declined in the tissues of Phaseolus vulgaris L. plants decreased with increasing concentrations of heavy metals (Pb and Cd).

### Table 2

<table>
<thead>
<tr>
<th>Number of leaves</th>
<th>Leaf length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Chlorophyll (mg/cm²)</th>
<th>Carbohydrate content (%)</th>
<th>Lipid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose 0</strong></td>
<td>30.0 ± 0.7ab</td>
<td>13.4 ± 0.7a</td>
<td>91 ± 11a</td>
<td>12 ± 1.8b</td>
<td>0.016 ± 0.004a</td>
<td>2.1 ± 0.6a</td>
</tr>
<tr>
<td><strong>Dose 1</strong></td>
<td>29.6 ± 0.9b</td>
<td>13.1 ± 0.8b</td>
<td>82 ± 5b</td>
<td>11 ± 0.5b</td>
<td>0.016 ± 0.004b</td>
<td>2.6 ± 0.7b</td>
</tr>
<tr>
<td><strong>Dose 2</strong></td>
<td>26.6 ± 2.9b</td>
<td>13.1 ± 0.7a</td>
<td>83 ± 8b</td>
<td>11 ± 0.4b</td>
<td>0.016 ± 0.005a</td>
<td>0.9 ± 0.6b</td>
</tr>
<tr>
<td><strong>Dose 3</strong></td>
<td>28.2 ± 1.1b</td>
<td>13.0 ± 0.8a</td>
<td>79 ± 6b</td>
<td>11 ± 1.0b</td>
<td>0.015 ± 0.005b</td>
<td>0.8 ± 0.6b</td>
</tr>
<tr>
<td><strong>Dose 4</strong></td>
<td>26.0 ± 2.9b</td>
<td>12.4 ± 0.7a</td>
<td>68 ± 10b</td>
<td>9 ± 0.9a</td>
<td>0.015 ± 0.004c</td>
<td>0.7 ± 0.4a</td>
</tr>
</tbody>
</table>

*Plants did not survive at Dose 5, dying at day 29 from planting. Different letters show statistical differences between doses (p < 0.05).
as the AB concentration in the pig slurry increased, although the change was only statistically significant at the highest AB dose. This is consistent with the fact that plants exposed to abiotic stress (temperature, salinity, or nutrient depletion) tend to accumulate lipids (Singer et al., 2016). In fact, phospholipid signaling is an important component of the early response of plant cells to environmental changes (Xue et al., 2007).

### 3.4. Metabolic response

The metabolomic data show two types of responses, as seen in the hierarchical clustering heatmap of the features (Fig. 3): D0, D1, and D2 form one cluster, while D3 and D4 from another. In the first group (D0, D1, and D2), one-way ANOVA found no features with significant differences (adjusted p-value < 0.05). For the second group (D3, D4), the data indicate that lettuces exposed to D3 and D4 had a different type of response, probably due to stress. The responses to D3 and D4 were similar, with no features with statistical differences (adjusted p-value < 0.05). Fig. 3 shows that 4-hydroxybenzoic acid increased slightly from D0 to D2 and then dramatically decreased at D3 and D4. This compound is a phenolic acid and has been linked to different types of responses to abiotic stresses (Samec et al., 2021). This fact reinforces the idea that a different level of stress was triggered after D3.

Volcano plots (FC > 1.5 and p-value < 0.05) (Fig. 3-SM and Table 2-SM) were made to compare each dose to D0 (control). No features were highlighted between D0 and D1. However, between D0 and D2, 15 metabolites were highlighted as significantly down-regulated, including rhamnose, a metabolite which is involved on the central carbon metabolism. This is in line with the decrease in carbohydrate content found in D2 lettuces (Table 2), as well as with the fact that D2 was the first dose to result in AB uptake (only sulfadiazine). Thus, the data indicate that pig slurry containing 0.5 mg/kg fw of ABs (D2) already had an effect on lettuce metabolism.

As for the comparison of D0 to D3, the volcano plot showed that 6 metabolites were highlighted as significantly down-regulated (decreased in concentration in comparison to D0, control) and 10 as up-regulated (increased in concentration in comparison to D0, control). Most of these metabolites had an FC > 2, indicating important concentration changes. Finally, the comparison of D0 vs D4 revealed only a significant up-regulation of 4 metabolites. Based on this, the metabolites that were significantly up-regulated or down-regulated between D0 and D3 (Fig. 3-SM) were plotted on a central carbon diagram to show the implications of the dose resulting in the greatest number of metabolites affected. Of these features, five could be related to the central carbon metabolism (L-rhamnose, 4-aminobutanoic acid, serine, norleucine, and diethanolamine). Interestingly, 4-aminobutanoic acid, which has been reported as a signaling molecule under plant stress (Kaspal et al., 2021), was dramatically down-regulated in the D3 samples (Table 2-SM). In fact, in a previous study by the present group, 4-aminobutanoic acid was up-regulated in lettuces fertilized with organic wastes (Matamoros et al., 2021). Instead, L-rhamnose was up-regulated and serine, norleucine, and diethanolamine were down-regulated in the D3 samples. Such a pattern was likewise observed in previous research in which lettuces were fertilized with organic fertilizers (Matamoros et al., 2021). Additionally, a feature assigned to the plant steroid pregnan-11-one was much higher at D3 (and D4) than at D0 (Fig. 3 and 3-SM). As plant steroids are related to plant growth, development, and stress (Vriet et al., 2013), the up-regulation of such a feature supports the idea that D3 is triggering plant stress.

The aforementioned previous study found that AB concentrations measured in lettuces fertilized with native organic wastes (containing 1–52 µg/g of ABs fw) did not correlate with any metabolic features (Matamoros et al., 2021). However, that study examined only one (native) dose. Our findings suggest that organic waste fertilizers containing specific AB doses (in this case, D3, with 5 mg/kg fw) can already induce plant stress. All in all, given the changes in the lettuce metabolisms, the current findings indicate that, while only the D4 samples were compromised agronomically and showed incorporation of other ABs besides sulfadiazine, the D2 dose already triggered a metabolic response.
from the lettuce, and D3 was probably inducing plant stress.

3.5. Human health implications

Human health risk was assessed by taking into consideration the HQ, as well as the legislated values for ABs in foodstuffs established by the pertinent EU Council Regulation. The AB concentration levels in lettuce edible parts (Table 2) were less than the MRLs established for animal tissues or food samples by EU regulations (The European Commission, 2010), ranging from 100 to 500 μg/kg for fluoroquinolones, 100–600 μg/kg for tetracyclines, and 100 μg/kg for sulfonamides, depending on the foodstuff.

Table 3 shows that while the HQ does depend on the AB dosage in the pig slurry, even at the greatest dosage measured (D4, 50 mg/kg of each AB), the risk was much lower than 0.1. Sulfadiazine was the AB found at the greatest concentration in the lettuce edible parts and thus the one with the greatest HQ. Even so, the THQ was lower than 0.01 in all the study scenarios, indicating that the intake of lettuce fertilized with pig slurry does not pose a human health risk. Similar results have been reported by other authors following crop fertilization with manure (Margenat et al., 2020). Nevertheless, the presence of these ABs in vegetables following pig slurry fertilization may pose an indirect threat to human health by promoting the selection of pathogens carrying AMR elements (Sorinolu et al., 2021). Furthermore, this study has the limitation of only taking into consideration lettuce intake, so potential human health risk from the intake of ABs from other crops or drinking water cannot be disregarded.

4. Conclusions

This study shows that the use of pig slurry in agriculture results in increased AB uptake by plants as the concentration of ABs increases in the fertilizers but does not pose a direct human health risk. The following conclusions can be drawn:

- Lettuce uptake of ABs from pig slurry was only observed at a dose of 0.5 mg/kg fw (D2) for sulfadiazine: the other ABs were only detected in lettuce at higher AB concentrations in the fertilizer.
- Sulfadiazine was the AB found at the highest concentration level in lettuce leaves (58 mg/kg fw), but only lincomycin TPs (hydroxy/sulfate derivatives) were found in crops at D4 (50 mg/kg).
- Increases in the dose of ABs in the pig slurry resulted in changes in fresh weight and lipid and carbohydrate content, probably due to the reduction of nitrogen assimilation.

-Metabolomic changes were observed at D2 (0.5 mg/kg fw) and higher. However, the most pronounced changes (number of metabolites and FC) were seen at D3 (5 mg/kg fw).

- The presence of ABs in lettuce following pig-slurry fertilization does not pose a direct human health risk. Nevertheless, human health exposure to low AB concentrations can promote AMR selection.

Nevertheless, to fully understand the effects of ABs in agriculture, additional field-scale investigations are required, including the repeated application of manure containing high concentrations of ABs and the monitoring of AB TPs in crop tissues.

Author contribution statement


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.

Acknowledgments

The authors gratefully acknowledge the financial support of the Spanish Ministry of Science and Innovation through project AGL 2017-89518-R. IDAEA-CSIC is a Severo Ochoa Centre of Excellence (Spanish Ministry of Science and Innovation, Project CEX 2018-000794-S). Mónica Escolà Casas wishes to thank the Beatriz de Pinós 2018 grant program (MSCA grant agreement number 801370) for the funding. The authors likewise thank Miquel Massip, Daniel Fenero, and Nerea Granados for their technical assistance in the greenhouse facility.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114237.

References


V. Matamoros et al.

Environmental Research 215 (2022) 114237

7