**Supplementary materials**

**Assessment of the combined inputs of antimicrobials from top soil improvers and irrigation waters on green leafy vegetables fields.**

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**Table 1 SM:** Extraction procedures of antimicrobials from the considered matrices

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| --- | --- | --- | --- |
|  | | | |
| **Step** | **Irrigation watera** | **Slurries and digestates** | **Top soil improversa** |
| Sample weight | 20 mL | 1.5 g | 0.5 g |
| Extraction – 1 | 10 µL Na2EDTA 0.1 M pH = 3 | 7 mL ACN/H2O 80/20 (v/v) containing HCOOH 0.5% and 390 mg di Na2EDTA x 2H2O | 15 mL McIlvane buffer pH = 7 containing 0.1 M of Na2EDTA / MeOH 40/60 v/v |
| Extraction – 2 |  | 3 mL of ACN/H2O 80:20 v/v, containing 1 % of NH4OH | 5 mL of Na2EDTA 0.1 M and 10 mL McIlvane buffer pH = 3 / MeOH 40/60 v/v |
| Extraction – 3 |  |  | 5 mL Na2EDTA 0.1 M and 10 mL of ammonium chloride/ammonia pH = 10 / MeOH 40/60 v/v |
| Extraction – 4 |  |  | Evaporation of organic phase, dilution and pH adjustment to 3 |
| SPE clean-up | OASIS HLB/Strata X-C | NH2 | OASIS HLB/Strata X-C |
| SPE conditioning – 1 | 6 mL MeOH | 6 mL ACN | 6 mL MeOH |
| SPE conditioning – 2 | 6 mL CH3COOH 0.1% |  | 6 mL CH3COOH 0.1% |
| Sample loading | Discard | Collect | Discard |
| Wash | 6 mL of water |  | 6 mL of water |
| Elution – 1 | 3 mL ACN/NH4OH 30% 70/30 v/v (Strata X-C) | 1.5 mL of ACN | 3 mL ACN/NH4OH 30% 70/30 v/v (Strata X-C) |
| Elution – 2 | 3 mL MeOH/NH4OH 30% 70/30 v/v (Strata X-C) |  | 3 mL MeOH/NH4OH 30% 70/30 v/v (Strata X-C) |
| Elution – 3 | 6 mL MeOH |  | 6 mL MeOH |
| Evaporation | 40°C under nitrogen stream | 40°C under nitrogen stream | 40°C under nitrogen stream |
| Final solvent | 200 µL ammonium acetate 0.2M | 1.5 mL ammonium acetate 0.2M / ACN 70/30 v/v | 1.5 mL of ammonium acetate 0.2M |
| Centrifugation | 12000 rpm for 5 min. | 12000 rpm for 5 min. | 12000 rpm for 5 min. |

aSargenti et al. (2020)

**Table 2 SM**. Retention Times (RT) and Relative Retention times (RRT) along with the m/z ions monitored for the considered antimicrobials

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **N°** | **Analytes** | **IS** | **RT**  **(min)** | **RRT** | **Precursor (*m/z*)** | **Adduct** | **Ion 1**  **(*m/z*)** | **Ion 2**  **(*m/z*)** |
| 1 | Sulfaguanidine | Sulfanilammide-13C6 | 3.20 | 0.89 | 215.0597 | [M+H]+ | 156.0 | 108.1 |
| 2 | Sulfanilammide | Sulfanilammide-13C6 | 3.60 | 1.00 | 156.0114 | [M+H]+ | 92.1 | 108.1 |
|  | Sulfanilammide-13C6 | - | 3.60 | - | - | [M+H]+ | - | - |
| 3 | Florfenicolamina | Florfenicolo-d3 | 3.60 | 0.35 | 230.0646 | [M+H]+ | 230.1 | 130.1 |
| 4 | Sulfadiazine | Sulfametazine -13C6 | 6.70 | 0.74 | 251.0597 | [M+H]+ | 156.0 | 108.1 |
| 5 | Sulfathiazole | Sulfametazine -13C6 | 7.30 | 0.81 | 256.0209 | [M+H]+ | 156.0 | 108.1 |
| 6 | Sulfapyridine | Sulfametazine -13C6 | 7.75 | 0.86 | 250.0645 | [M+H]+ | 156.0 | 184.1 |
| 7 | Tildipirosina | Spiramycin-I-d3 | 7.70 | 0.65 | 245.5153 | [M+3H]+++ | 245.5 | 281.2 |
| 8 | Sulfamerazine | Sulfametazine -13C6 | 7.95 | 0.88 | 265.0754 | [M+H]+ | 156.0 | 190.0 |
| 9 | Lincomycin | Spiramycin-I-d3 | 8.50 | 0.71 | 407.2210 | [M+H]+ | 407.2 | 126.1 |
| 10 | Trimethoprim | Sulfametazine -13C6 | 8.65 | 0.96 | 291.1451 | [M+H]+ | 230.1 | 261.1 |
| 11 | Tulathromycin marker (CP 60.300) | Spiramycin-I-d3 | 8.70 | 0.73 | 289.2066 | [M+2H]++ | 289.2 | 420.3 |
| 12 | Thiamphenicol | Florfenicolo-d3 | 8.70 | 0.84 | 356.0121. 373.0386 | [M+H]+ | 308.0 | 338.0 |
| 13 | 4-epi-tetracycline | Metacycline | 8.70 | 0.91 | 445.1605 | [M+H]+ | 267.1 | 201.1 |
| 14 | Marbofloxacina | Enrofloxacin-d5 | 8.75 | 0.89 | 363.1463 | [M+H]+ | 320.1 | 363.1 |
| 15 | Sulfametazine | Sulfametazine -13C6 | 9.05 | 1.00 | 279.0910 | [M+H]+ | 124.1 | 204.0 |
|  | Sulfametazine -13C6 | - | 9.05 | - | - | [M+H]+ | - | - |
| 16 | 4-epi-ossitetracycline | Metacycline | 9.50 | 0.72 | 461.1555 | [M+H]+ | 201.1 | 337.1 |
| 17 | Tetracycline | Metacycline | 9.60 | 0.73 | 445.1605 | [M+H]+ | 269.1 | 241.1 |
| 18 | Norfloxacin | enrofloxacin-d5 | 9.72 | 0.98 | 320.1405 | [M+H]+ | 320.1 | 276.2 |
| 19 | Ossitetracycline | Metacycline | 9.80 | 0.75 | 461.1555 | [M+H]+ | 201.1 | 337.1 |
| 20 | Ciprofloxacin | Enrofloxacin-d5 | 9.80 | 0.99 | 332.1405 | [M+H]+ | 332.1 | 245.1 |
| 21 | Enrofloxacin | Enrofloxacin-d5 | 9.85 | 1.00 | 360.1718 | [M+H]+ | 203.1 | 245.1 |
|  | Enrofloxacin-d5 | - | 9.85 | - | - | [M+H]+ | - | - |
| 22 | Tulathromycin | Spiramycin-I-d3 | 9.90 | 0.83 | 269.5295 | [M+3H]+++ | 269.5 | 289.2 |
| 23 | Danofloxacin | Enrofloxacin-d5 | 10.00 | 1.02 | 358.1561 | [M+H]+ | 358.2 | 255.1 |
| 24 | Sulfamethoxazole | Sulfametazine -13C6 | 10.15 | 1.21 | 254.0594 | [M+H]+ | 156.0 | 254.0 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **N°** | **Analytes** | **IS** | **RT**  **(min)** | **RRT** | **Precursor (*m/z*)** | **Adduct** | **Ion 1**  **(*m/z*)** | **Ion 2**  **(*m/z*)** |
| 25 | Difloxacin | Enrofloxacin-d5 | 10.30 | 1.05 | 400.1467 | [M+H]+ | 400.1 | 356.2 |
| 26 | Sulfamonomethoxine | Sulfametazine -13C6 | 10.40 | 1.15 | 281.0703 | [M+H]+ | 156.0 | 108.1 |
| 27 | Florfenicol | Florfenicolo-d3 | 10.40 | 1.00 | 358.0077. 375.0343 | [M+H]+ | 340.0 | 241.0  358.0 |
|  | Florfenicolo-d3 | - | 10.40 | - | - | [M+H]+ | - | - |
| 28 | Sarafloxacin | Enrofloxacin-d5 | 10.60 | 1.08 | 386.1311 | [M+H]+ | 299.1 | 386.1 |
| 29 | 4-epi-clortetracycline | Metacycline | 11.00 | 0.84 | 479.1216 | [M+H]+ | 303.0 | 371.0 |
| 30 | Neospiramycin | Spiramycin-I-d3 | 11.30 | 0.95 | 350.2250 | [M+2H]++ | 174.1 | 160.1 |
| 31 | Clortetracycline | Metacycline | 11.90 | 0.90 | 479.1216 | [M+H]+ | 303.0 | 275.0 |
| 32 | Spiramycin | Spiramycin-I-d3 | 11.90 | 1.00 | 422.2643 | [M+2H]++ | 422.3 | 540.3 |
|  | Spiramycin-I-d3 | - | 11.90 | - | - | [M+2H]++ | - | - |
| 33 | Sulfadimethoxine | Sulfametazine -13C6 | 12.40 | 1.37 | 311.0809 | [M+H]+ | 156.0 | 108.1 |
| 34 | Sulfaquinoxaline | Sulfametazine -13C6 | 12.85 | 1.42 | 301.0754 | [M+H]+ | 156.0 | 108.1 |
|  | Oxolinic acid | Enrofloxacin-d5 | 13.05 | 1.32 | 262.0710 | [M+H]+ | 160.0 | 234.1 |
| 35 | Metacycline | - | 13.15 | - | - | [M+H]+ | - | - |
| 36 | Gamithromycin | Spiramycin-I-d3 | 13.25 | 1.11 | 777.5471; 389.2772 | [M+H]+; [M+2H]++ | 777.5  619.5 | 619.5  389.3 |
| 37 | Tilmicosina | Spiramycin-I-d3 | 13.40 | 1.13 | 435.2900 | [M+2H]++ | 695.5 | 435.3 |
| 38 | Doxycycline | Penicillina G-d7 | 13.55 | 0.90 | 445.1605 | [M+H]+ | 267.1 | 321.1 |
| 39 | Nalidixic acid | Enrofloxacin-d5 | 14.80 | 1.49 | 233.0921 | [M+H]+ | 233.1 | 205.1 |
| 40 | Tiamulina | Spiramycin-I-d3 | 15.10 | 1.27 | 494.3299 | [M+H]+ | 192.1 | 494.3 |
| 41 | Flumequine | Enrofloxacin-d5 | 15.25 | 1.55 | 262.0874 | [M+H]+ | 238.1 | 262.1 |
| 42 | Tylosin A | Spiramycin-I-d3 | 15.35 | 1.29 | 363.7248 | [-ossano+2H]++ | 174.1 | 336.2 |
| 43 | Erythromycin A | Spiramycin-I-d3 | 15.60 | 1.31 | 734.4685 | [M+H]+ | 576.4 | 734.5 |
| 44 | 3-O-acetiltylosin | Spiramycin-I-d3 | 15.70 | 1.32 | 384.7301 | [-ossano+2H]++ | 109.1 | 174.1 |
| 45 | Anhydrous erythromycin | Spiramycin-I-d3 | 16.20 | 1.10 | 716.4580 | [M+H]+ | 558.4 | 158.2 |
| 46 | Valnemuline | Spiramycin-I-d3 | 17.20 | 1.42 | 565.6370 | [M+H]+ | 565.4 | 263.1 |
| 47 | Tylvalosin | Spiramycin-I-d3 | 17.40 | 1.46 | 426.7588 | [-ossano+2H]++ | 109.1 | 174.1 |
| 48 | Rifaximin | spiramycin-I-d3 | 18.50 | 1.55 | 786.3596 | [M+H]+ | 754.4 | 362.1 |

**Table 3 SM.** LODs (ng/g wet weight) for the selected antimicrobials in the different types of top soil improvers

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Class** | **Analytes** | **Sludge from cheese plant** | **Mixed Compost** | **Sludge from WWTP** | **Sludge with lime** | **Digestates** | **Bovine manure** | **Pig Slurry** |
| **Lincosamides** | Lincomycin | 25 | 25 | 25 | 50 | 10 | 10 | 10 |
| **Quinolones** | all | 25 | 25  (Ciprofloxacin 100) | 25 | 25 | 10 | 10 | 10 |
| **Tetraciclines** | all | 25 | 25 | 25 | 25  (Doxicicline 50) | 10 | 10 | 10 |
| **Pleuromutilines** | Tiamulina | 25 | 25 | 25 | 25 | 10 | 10 | 10 |
| Valnemuline |  |
| **Rifacimines** | Rifaximin | 25 | 25 | 25 | 50 | 10 | 10 | 10 |
| **Sulfonamides** | All | 25 | 25 | 25 | 25  (sulfanilammide 250) | 10 | 10 | 10 |
| **Amfenicoles** | Florfenicol-  amine | 25 | 25 | 25 | 50 | 25 | 25 | 10 |
| Thiamphenicol |  | 50 |  | 500 |  |  |  |
| Florfenicol |  | 50 |  | 500 |  |  |  |
| **Macrolides** | Tildipirosina | 25  (Erythromycin 250) | NAa | 25  (Erythromycin 250) | 25 | 10 | 10 | 10 |
| CP60300 | 25 | 25 |
| Neospiramycin | 25 | 25 |
| Tulathromycin | NAa | 25 |
| Gamithromycin | 25 | 25 |
| Spiramycin | 25 | 25 |
| Tilmicosin | 25 | 25 |
| Erythromycin A | 100 | 250 |
| Erythromycin A anidra | 25 | 25 |
| Tylosin A | 25 | 50 |
| 3-O-acetyltylosin | 25 | 25 |
| Tylvalosin | 25 | 100 |

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Class** | **Analytes** | **Bovine manure aNA bone meals** | **Mixed animal manure aNA green compost** | **Mixed compost aNA minerals** | **Mixed Compost** | **Bovine manure from organic farm** | **Poultry litter** |
| **Sulfonamides** | all | 25  (sulfanilammide 50) | 25  (sulfanilammide 50) | 25  (sulfanilammide 50) | 25  (sulfanilammide 50) | 10 | 25  (sulfaguanidine 50)  (sulfanilammide 250) |
| **Amfenicoles** | Florfenicolamina | 25 | 25 | 25 | 25 | 25 | 50 |
| Thiamphenicol | 50 | 50 | 50 | 50 | 500 |
| Florfenicol | 100 | 100 | 100 | 100 | 500 |
| **Macrolides** | Tildipirosina | NAa | NAa | NAa | NAa | 10 | 50 |
| CP60300 | 25 | 25 | 25 | 25 | 25 |
| Neospiramycin | 25 | 25 | 25 | 25 | 100 |
| Tulathromycin | 500 | 500 | 500 | 500 | 100 |
| Gamithromycin | 25 | 25 | 25 | 25 | 25 |
| Spiramycin | 25 | 25 | 25 | 25 | 25 |
| Tilmicosina | 25 | 25 | 25 | 25 | 25 |
| Erythromycin A | 25 | 25 | 25 | 25 | 250 |
| Erythromycin A Anhydrous | 25 | 25 | 25 | 25 | 25 |
| Tylosin A | 25 | 25 | 25 | 25 | 25 |
| 3-O-acetyltylosin | 25 | 25 | 25 | 25 | 250 |
| Tylvalosin | 25 | 25 | 25 | 25 | 100 |
| **Lincosamides** | Lincomycin | 25 | 25 | 25 | 25 | 10 | 25 |
| **Quinolones** | all | 25 | 25 | 25 | 25 | 10 | 25 |
| **Tetraciclines** | all | 25  (Doxycycline 50) | 25  (Doxycycline 50) | 25  (Doxycycline 50) | 25  (Doxycycline 50) | 10 | 25 |
| **Pleuro-mutilines** | all | 25 | 25 | 25 | 25 | 10 | 25 |
| **Rifacimines** | Rifaximin | 100 | 100 | 100 | 100 | 10 | 25 |

NA = not analysable; tildipyrosine was not analysable in samples No. 1.0, 2.0, 3.0, 4.0, 5.0, 5.2, 6.0, 11, 14, and 19; tulatromycine was not analysable in samples No. 5.0, 5.2, 6.0, 11, 14, and 19.

**Table 4 SM.** LODs of antimicrobial classes in irrigation waters.

|  |  |
| --- | --- |
| **Class** | **LOD (ng/L)** |
| Sulfonamides | 10 |
| Amfenicoles | 10 |
| Macrolides | 10 |
| Lincosamides | 10 |
| Quinolones | 10 |
| Tetracyclines | 10 |
| Pleuromutilines | 10 |
| Rifacimines | 10 |
| β-lactams | 10 |