To be submitted to

Occurrence of antibiotics in lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) following organic soil fertilization: Crop and human health implications

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Number of Tables: **10**

Number of Figures: **1**

**Reagents and materials**

8-Hydroxyquinoline, sulfacetamide, sulfapyridine, sulfathiazole, sulfamethizole, sulfadiazine, sulfamethazine, sulfamethoxazole, lincomycin, ciprofloxacin, ofloxacin (OFL), enrofloxacin (ENR), ofloxacin-d3 (OFL-d3), enrofloxacin-d5 hydrochloride (ENR-d5), trimethoprim, oxytetracycline, tetracycline, and doxycycline were purchased from Sigma Aldrich (St. Louis, MO, USA). OFL Methyl Ester, OFL Ethyl Ester, decarboxyl OFL (OFL impurity B), N-desmethyl OFL (OFL impurity E), keto TMP (TMP impurity B) (2,4-diaminopyrimidin-5-yl)-(3,4,5-trimethoxyphenyl)methanone, TMP306 (2,4-diaminopyrimidin-5-yl)-(3,4,5-trimethoxyphenyl)methanol (hydroxyl TMP = TMP impurity C) and N-Acetyl SMX (SMX impurity A) were purchased from LGC standards S.L.U. (Barcelona, Spain). Trimethoprim-d3 (TMP-d3) was purchased from Dr. Ehrenstorfer (Augsburg, Germany), and sulfamethoxazole-d4 (SMX-d4) was purchased from Analytical Standard Solutions – A2S (Saint Jean d’Illac, France), respectively. All standards were high-purity (95% or higher). Individual stock solutions were prepared at a concentration of 200 µg/mL in methanol. The stock solutions were stored in the freezer at −20ºC. Methanol and water (both LC-MS grade), ethyl acetate (GC-ECD/FID grade), analytical-grade fuming hydrochloric acid (37%) and formic acid (98-100%, pro analysis) were obtained from Merck (Darmstadt, Germany), whereas acetonitrile (LC-MS grade) was obtained from Fisher Scientific UK (Loughborough, UK). SPE Strata-X cartridge (100 mg 6 m L-1) were obtained from Phenomenex (Torrance, CA, USA) and 0.22 µm pore nylon filter were purchased from Sigma Aldrich (St. Louis, MO, USA).

**Determination of Antibiotics in vegetable samples**

100 µL of a surrogate standard (1µg/mL) was added in 1 g wet weight sample and left to equilibrate for 1h. A vegetable sample was then extracted with 10 mL of methanol in an ultrasonic bath (35 kHz) for 15 min. The extracts were centrifuged for 15 min at 3000 rpm. Two extraction cycles were required. The supernatants were decanted and combined in a glass vial and evaporated under a gentle nitrogen at a temperature of 40ºC until they had been reduced to 1 mL. The concentrated extracts were then diluted with 10 mL of water to perform the SPE clean-up step with Strata-X (100mg/6mL). Then SPE was first preconditioned with 6 mL methanol and 6 mL H2O. After sample loading, the polymeric cartridge was then dried under a nitrogen stream, followed by elution with 2 mL of a mixture of methanol and ethyl acetate (50/50, v/v). The eluted fraction was evaporated to dryness and reconstituted in 1 mL of mobile phase. Then the final extracts were filtered through a 0.22 µm pore nylon filter and injected into HPLC-MS/MS.

**Plant metabolomics**

The GC injector temperature was set at 280 ºC. The oven temperature was set at 70 ºC and increased to 100 ºC at a rate of 7 ºC/min, then to 260 ºC at a rate of 5 ºC/min, and finally to 300 ºC at a rate of 10 ºC/min. The applied electron ionization energy was 70 eV, and the transfer line and ion source were set at 280 °C and 260 °C respectively. Scanning was performed from 50 to 650 m/z at a mass resolution of 60,000 (full width at half maximum (FWHM) at m/z 200) for non-target analysis of lettuce sample extracts.

**Metabolomics workflow**

Non-target GC-Orbitrap data were processed with Xcalibur 4.1 and TraceFinder 4.0 software (Thermo Scientific). TraceFinder 4.0 plug-in allowed peak detection, spectral deconvolution, peak alignment and library search. A signal-to-noise ratio cut-off of 50, a mass tolerance of 5 ppm, minimum TIC intensity of 1 x 105 and an ion overlap window of 98% were used. All ions in the deconvoluted spectra between 11 and 45 minutes were used for the subsequent library search (in NIST) of candidate compounds. In the following step of the workflow, the tentative hits returned for each peak were scored based on the classical search index (SI) score and high-resolution filtering (HRF) value. HRF index indicates the percentage of the high resolution spectrum that can be explained by the chemical formula proposed from the best library match result. Thus, all hits with SI over 600 and a correspondent HRF over 90 were selected for further data analysis. In addition, peak selection and integrations were manually validated.

**Table 1-SM**. Chemical composition in dry weight of fertilization products. Samples were analysed in triplicate. The amount of fertilization product added per pot was calculated to ensure the same quantity of total nitrogen in all treatments (100 kg of N per ha).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | SS | PS | OFMSW | CF\* |
| Kjeldahl Nitrogen (g kg-1) | 27.7±4.4 | - | 26.1±4.2 | 340 |
| Ammonia-nitrogen (g kg-1) | 13.4±0.9 | 48±1.5 | 2.9±0.2 | 170 |
| Nitrate-nitrogen (g kg-1) | <0.1 | 0.5±0.1 | 0.4±0.1 | 170 |
| P (g kg-1, acid extraction) | 15.5±6.2 | 6.1±2.1 | 5.5±2.2 | 190 |
| K (g kg-1, acid extraction) | 21.0±5.5 | - | 24.2±6.3 | 430 |
| Moisture content (%) | 80% | 96% | 23% | - |

\*Chemical fertilizer composition was as follows: ammonium nitrate (34% N), phosphate (44% P2O5) and potassium sulfate (52% K2SO4).

**Table 2-SM.** Analytical quality parameters for the determination of antibiotics in fertilization products.

|  |  |  |
| --- | --- | --- |
| **Compound** | **LOD [ng/g fw]** | **LOQ [ng/g fw]** |
| Lincomycin | 2.44 | 5.53 |
| Sulfacetamide | 0.07 | 0.14 |
| Sulfadiazine | 0.07 | 0.14 |
| Sulfapyridine | 0.90 | 2.44 |
| Oxytetracycline | 2.34 | 3.07 |
| Sulfathiazole | 1.14 | 2.53 |
| Tetracycline | 2.01 | 3.12 |
| Sulfamethazine | 0.57 | 1.38 |
| Ofloxacin | 0.73 | 1.61 |
| Ciprofloxacin | 0.77 | 1.49 |
| Enrofloxacin | 1.23 | 2.97 |
| Sulfamethizole | 1.09 | 3.09 |
| Doxycycline | 1.50 | 1.82 |
| Sulfamethoxazole | 0.91 | 2.23 |

**Table 3-SM.** Analytical quality parameters for the determination of antibiotics in lettuce samples.

|  |  |  |
| --- | --- | --- |
| **Compound** | **LOD [ng/g fw]** | **LOQ [ng/g fw]** |
| 8-Hydroxyquinoline\* | 0.13 | 0.68 |
| Sulfacetamide | 1.33 | 1.62 |
| Trimethoprim | 0.65 | 2.08 |
| Hydroxyl-trimethoprim\* | 0.59 | 1.93 |
| Decarboxyl-ofloxacin \* | 1.01 | 3.74 |
| Ofloxacin Methyl Ester\* | 0.71 | 0.72 |
| Lincomycin | 0.19 | 0.21 |
| Sulfadiazine | 0.25 | 1.66 |
| Sulfapyridine | 0.26 | 0.68 |
| Sulfathiazole | 0.68 | 0.76 |
| Sulfamethazine | 1.33 | 1.59 |
| Keto-trimethoprim\* | 0.30 | 0.85 |
| Ciprofloxacin | 0.19 | 0.31 |
| N-desmethyl Ofloxacin\* | 0.34 | 0.63 |
| Ofloxacin | 0.89 | 3.27 |
| Ofloxacin Ethyl Ester\* | 0.56 | 1.60 |
| Enrofloxacin | 1.23 | 1.37 |
| Sulfamethizole | 1.28 | 1.82 |
| Sulfamethoxazole | 0.73 | 0.79 |
| N-Acetyl-sulfamethoxazole\* | 0.61 | 0.63 |
| Oxytetracycline | 0.71 | 0.99 |
| Tetracycline | 0.15 | 1.64 |
| Doxycycline | 0.47 | 0.65 |

\*Transformation products

**Table 4-SM.** Analytical quality parameters for the determination of antibiotics in radish samples.

|  |  |  |
| --- | --- | --- |
| **Compound** | **LOD [ng/g fw]** | **LOQ [ng/g fw]** |
| 8-Hydroxyquinoline\* | 0.66 | 1.93 |
| Sulfacetamide | 2.00 | 2.01 |
| Trimethoprim | 0.23 | 0.24 |
| Hydroxyl-trimethoprim\* | 0.59 | 0.79 |
| Decarboxyl-ofloxacin \* | 0.64 | 0.92 |
| Ofloxacin Methyl Ester\* | 0.47 | 1.07 |
| Lincomycin | 0.14 | 0.44 |
| Sulfadiazine | 0.47 | 0.73 |
| Sulfapyridine | 1.17 | 1.30 |
| Sulfathiazole | 1.08 | 1.16 |
| Sulfamethazine | 0.60 | 0.69 |
| Keto-trimethoprim\* | 0.35 | 0.41 |
| Ciprofloxacin | 1.86 | 3.56 |
| N-desmethyl Ofloxacin\* | 0.38 | 1.55 |
| Ofloxacin | 0.89 | 1.13 |
| Ofloxacin Ethyl Ester\* | 0.49 | 0.52 |
| Enrofloxacin | 0.36 | 1.66 |
| Sulfamethizole | 0.93 | 2.01 |
| Sulfamethoxazole | 0.69 | 0.77 |
| N-Acetyl-sulfamethoxazole\* | 0.34 | 0.42 |
| Oxytetracycline | 0.54 | 1.82 |
| Tetracycline | 0.44 | 0.48 |
| Doxycycline | 0.69 | 0.77 |

\*Transformation products

Table 5-SM. List of the features with SI >600 and HRF >90. The name is presented as given in the NIST hit. The table also includes the correspondent RSI and the monoisotopic mass of the main peak with high resolution. These features have been selected for data analysis and interpreation.

|  |  |  |  |
| --- | --- | --- | --- |
| **Peak @ “Retention Time” “m/z”\_Match Name** | **RSI/Rev Dot** | **SI/Dot Product** | **MonoIsotopic Mass** |
| peak @ 15.96 103.06\_Butanoic acid, 2,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester | 605 | 605 | 103.057419 |
| peak @ 23.38 117.07\_D-(-)-Rhamnose, tetrakis(trimethylsilyl) ether, methyloxime (syn) | 609 | 609 | 117.0731 |
| peak @ 21.89 141.08\_Asparagine, O,O',N-tris(trimethylsilyl)- | 615 | 615 | 141.08429 |
| peak @ 39.40 361.17\_D-(+)-Trehalose, octakis(trimethylsilyl) ether | 620 | 620 | 361.16788 |
| peak @ 40.77 217.11\_D-Psicofuranose, pentakis(trimethylsilyl) ether (isomer 1) | 625 | 614 | 217.107239 |
| peak @ 29.54 318.15\_Scyllo-Inositol, 6TMS derivative | 633 | 633 | 318.149353 |
| peak @ 24.22 217.11\_Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)- | 649 | 643 | 217.10718 |
| peak @ 41.23 204.10\_Maltose, 8TMS derivative | 650 | 644 | 204.09938 |
| peak @ 23.57 229.11\_Aconitic acid, (E)-, 3TMS derivative | 651 | 651 | 229.107468 |
| peak @ 29.86 333.14\_Galactaric acid, (R,S,R,S)-, 6TMS derivative | 655 | 653 | 333.136505 |
| peak @ 41.39 204.10\_β-Gentiobiose, octakis(trimethylsilyl) ether, methyloxime (isomer 2) | 659 | 659 | 204.09938 |
| peak @ 42.21 204.10\_α-D-Lactose, 8TMS derivative | 671 | 647 | 204.09946 |
| peak @ 29.93 143.05\_Pentenoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester | 676 | 648 | 143.05229 |
| peak @ 26.26 345.17\_Quininic acid (5TMS) | 679 | 666 | 345.17276 |
| peak @ 28.28 79.05\_Glutaric acid, di((cyclohex-3-enyl)methyl) ester | 680 | 680 | 79.054245 |
| peak @ 25.85 264.11\_Adenine, 2TMS derivative | 682 | 682 | 264.10947 |
| peak @ 40.89 204.10\_β-Gentiobiose, octakis(trimethylsilyl) ether, methyloxime (isomer 1) | 683 | 683 | 204.09938 |
| peak @ 20.86 217.11\_Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)- | 687 | 673 | 217.107315 |
| peak @ 40.57 217.11\_D-(-)-Tagatofuranose, pentakis(trimethylsilyl) ether (isomer 1) | 691 | 688 | 217.10733 |
| peak @ 21.00 179.09\_4-Hydroxybenzeneacetic acid, 2TMS derivative | 692 | 631 | 179.088593 |
| peak @ 39.81 217.11\_Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)- | 698 | 685 | 217.10733 |
| peak @ 25.02 204.10\_Shikimic acid (4TMS) | 712 | 712 | 204.09938 |
| peak @ 29.58 204.10\_Ethyl α-D-glucopyranoside, 4TMS derivative | 715 | 706 | 204.099365 |
| peak @ 13.48 147.07\_Butanedioic acid, 2TMS derivative | 720 | 699 | 147.065582 |
| peak @ 40.40 217.11\_D-(-)-Tagatofuranose, pentakis(trimethylsilyl) ether (isomer 1) | 724 | 711 | 217.107239 |
| peak @ 20.77 204.10\_d-Glucose, 6-O-[6-deoxy-2,3,4-tris-O-(trimethylsilyl)-α-l-mannopyranosyl]-2,3,4,5-tetrakis-O-(trimethylsilyl)- | 725 | 609 | 204.09952 |
| peak @ 41.78 204.10\_Maltose, 8TMS derivative | 725 | 709 | 204.09938 |
| peak @ 14.63 188.13\_N(O)-isoBOC, mono O-TBDMS diethanolamine | 736 | 666 | 188.128342 |
| peak @ 19.31 163.06\_tert-Butyldimethylsilyl 3-mercaptopropanoate | 737 | 606 | 163.060501 |
| peak @ 12.15 116.05\_L-Serine, 2TMS derivative | 738 | 738 | 116.052574 |
| peak @ 32.42 204.10\_L-(+)-Rhamnopyranose, 4TMS derivative | 744 | 727 | 204.09938 |
| peak @ 12.77 130.07\_Diethanolamine, 2TMS derivative | 748 | 748 | 130.068314 |
| peak @ 17.68 84.04\_Pyroglutamic acid, TMS derivative | 748 | 748 | 84.04435 |
| peak @ 38.59 204.10\_D-(+)-Cellobiose, (isomer 1), 8TMS derivative | 756 | 750 | 204.09938 |
| peak @ 41.07 204.10\_D-(+)-Cellobiose, (isomer 1), 8TMS derivative | 770 | 748 | 204.09938 |
| peak @ 35.45 204.10\_1-Aminocyclopentanecarboxylic acid, 3-chloropropoxycarbonyl-, tridecyl ester | 772 | 741 | 204.09938 |
| peak @ 12.64 299.07\_Phosphonic acid, tri-(trimethylsilyl) derivative | 773 | 740 | 299.070984 |
| peak @ 25.64 174.11\_δ Amino levulinic acid tri-TMS | 776 | 678 | 174.112717 |
| peak @ 12.32 174.11\_Dopamine, 4TMS derivative | 780 | 763 | 174.112701 |
| peak @ 37.78 204.10\_1-Aminocyclopentanecarboxylic acid, 3-chloropropoxycarbonyl-, tridecyl ester | 781 | 655 | 204.099365 |
| peak @ 34.67 204.10\_Ethyl α-D-glucopyranoside, 4TMS derivative | 782 | 772 | 204.099365 |
| peak @ 14.24 245.07\_2-Butenedioic acid, (E)-, 2TMS derivative | 785 | 689 | 245.065735 |
| peak @ 30.91 352.14\_N,9-bis(Trimethylsilyl)-6-[(trimethylsilyl)oxy]-9H-purin-2-amine | 786 | 785 | 352.14352 |
| peak @ 18.52 232.12\_Aspartic acid, 3TMS derivative | 788 | 722 | 232.118164 |
| peak @ 19.17 158.14\_L-Norleucine, 2TMS derivative | 790 | 623 | 158.135895 |
| peak @ 18.87 120.08\_DL-Phenylalanine, TMS derivative | 801 | 801 | 120.080742 |
| peak @ 14.71 204.12\_L-Serine, 3TMS derivative | 803 | 796 | 204.12323 |
| peak @ 28.65 204.10\_L-(+)-Rhamnopyranose, 4TMS derivative | 812 | 811 | 204.099365 |
| peak @ 43.22 204.10\_L-Rhamnose, 4TMS derivative | 819 | 750 | 204.09944 |
| peak @ 15.52 116.09\_Alanine, N-trimethylsilyl-, trimethylsilyl ester | 821 | 622 | 116.08902 |
| peak @ 16.09 373.09\_Sebacic acid, 2TBDMS derivative | 823 | 814 | 373.089874 |
| peak @ 19.07 132.08\_4-Methyl-N-(1,2,3,4-tetrahydro-isoquinolin-1-ylmethyl)-benzenesulfonamide | 823 | 731 | 132.07934 |
| peak @ 20.81 218.10\_L-Phenylalanine, 2TMS derivative | 833 | 833 | 218.102432 |
| peak @ 13.04 158.14\_L-Isoleucine, 2TMS derivative | 837 | 837 | 158.135803 |
| peak @ 20.95 217.11\_α-D-(-)-Ribopyranose, 4TMS derivative | 837 | 815 | 217.107315 |
| peak @ 18.42 156.08\_L-5-Oxoproline, , 2TMS derivative | 840 | 831 | 156.083801 |
| peak @ 13.34 174.11\_Ethanolamine, 3TMS derivative | 872 | 872 | 174.112701 |
| peak @ 18.63 174.11\_4-Aminobutanoic acid, 3TMS derivative | 874 | 848 | 174.112701 |
| peak @ 19.79 142.10\_L-Proline (+CO2), 2TMS derivative | 883 | 738 | 142.10463 |
| peak @ 12.56 158.14\_Norleucine, N-trimethylsilyl-, trimethylsilyl ester | 891 | 830 | 158.135849 |
| peak @ 13.09 142.10\_L-Proline, 2TMS derivative | 908 | 908 | 142.10463 |
| peak @ 12.93 180.05\_Niacin, TMS derivative | 917 | 817 | 180.047348 |
| peak @ 23.28 174.11\_Putrescine, 4TMS derivative | 971 | 927 | 174.112686 |

**Table 6-SM.** ADI values for the ABs which shoed concentration levels above the LOQ.

|  |  |
| --- | --- |
|  | ADI\*\* \*(µg/Kg bw/day) |
| 8-Hydroxyquinoline | 50 |
| Trimethoprim | 4.2 |
| Hydroxyl-trimethoprim\* | 4.2\*\* |
| Decarboxyl Ofloxacin\* | 3.2\*\* |
| Ofloxacin Methyl Ester\* | 3.2\*\* |
| Lincomycin | 10 |
| Sulfadiazine | 20 |
| Sulfamethazine | 1.6 |
| Keto-trimethoprim\* | 4.2\*\* |
| Ciprofloxacin | 0.15 |
| N-desmethyl Ofloxacin\* | 3.2\*\* |
| Ofloxacin | 3.2 |
| Ofloxacin Ethyl Ester\* | 3.2\*\* |
| Enrofloxacin | 6.2 |
| Sulfamethoxazole | 130 |
| N-Acetyl-Sulfamethoxazole\* | 130\*\* |
| Oxytetracycline | 3 |
| Tetracycline | 3 |
| Doxycicline | 3 |

*\*Transformation products; \*\*parental compound ADI values were considered when TPs information was not available; \*\*\* ADI values reported from literature and EFSA website*

**Table 7-SM.** Data results from volcano analysis showing the FC (Factor Change as the ration between the fertilizer treatment over CF) and the p-value of metabolites. FC threshold has been set to >1.5 and <0.66, and p-value has been set to <0.05 (in bold). Both axes presented in a logarithmic scale.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **OFMSW vs CF** | | **PS vs CF** | | **SS vs CF** | |
|  | FC | p-value | FC | p-value | FC | p-value |
| **Guanine** | **0.28** | **2.39E-03** | 1.1386 | 5.93E-01 | 0.63965 | 2.13E-01 |
| **Proline** | **0.3** | **1.88E-06** | 0.80196 | 3.28E-01 | 0.61277 | 5.85E-02 |
| **m/z 188.12837\*** | **0.36** | **1.69E-03** | **0.53677** | **2.33E-02** | 0.61692 | 6.85E-02 |
| **5-Oxoproline** | **0.36** | **1.14E-02** | 0.72053 | 3.83E-01 | 0.65105 | 1.83E-01 |
| **DL-Phenylalanine** | **0.41** | **1.37E-04** | 0.80515 | 1.43E-01 | **0.63556** | **2.21E-02** |
| **L-Norleucine** | **0.42** | **2.56E-02** | 0.75345 | 3.45E-01 | 0.95957 | 8.95E-01 |
| **L-Serine** | **0.42** | **4.52E-03** | 0.65274 | 1.13E-01 | 0.66921 | 1.49E-01 |
| **Maltose** | **0.44** | **3.87E-04** | 0.78137 | 2.16E-01 | 0.76704 | 1.43E-01 |
| **Proline [+ CO2]** | **0.45** | **4.2E-03** | 0.81939 | 3.27E-01 | 1.045 | 8.6E-01 |
| **Alanine** | **0.45** | **3.43E-02** | 0.72292 | 2.79E-01 | 0.83674 | 5.61E-01 |
| **Scyllo-Inositol** | **0.46** | **1.26E-02** | 0.92358 | 6.95E-01 | 0.63152 | 9.82E-02 |
| **Norleucine** | **0.51** | **7.26E-05** | 0.81893 | 1.72E-01 | **0.61128** | **6.36E-03** |
| **Fumaric\_acid** | **0.54** | **1.56E-03** | 0.80545 | 1.61E-01 | 0.67692 | 3.17E-02 |
| **Adenine** | **0.56** | **4.81E-02** | 1.025 | 9.16E-01 | 0.80294 | 4.31E-01 |
| **L-Isoleucine** | **0.58** | **1.4E-03** | 0.90541 | 5.42E-01 | 0.70493 | 8.91E-02 |
| **P-toluenesulfonamide\*\*** | **0.62** | **3.9E-02** | 0.91794 | 7.66E-01 | 0.85869 | 4.95E-01 |
| **L-Phenylalanine** | **0.62** | **1.22E-02** | 1.0344 | 8.56E-01 | **0.58895** | **4.82E-02** |
| **Ethanolamine** | **0.62** | **1.3E-03** | 0.87537 | 2.36E-01 | **0.60576** | **2.44E-03** |
| **Ethyl alpha-D-glucopyranoside** | **0.65** | **2.37E-03** | 1.0499 | 4.9E-01 | 2.0298 | 8.93E-02 |
| **GABA** | **1.84** | **3.18E-03** | **1.8001** | **4.19E-03** | 1.3011 | 3.2E-01 |
| **Dopamine** | **1.98** | **9.1E-05** | **1.6042** | **1.28E-03** | **1.6953** | **6.28E-03** |
| **L-Rhamnose** | **2** | **1.74E-02** | 1.0784 | 7.51E-01 | 1.3455 | 1.77E-01 |
| **Rhamnopyranose** | 0.68696 | 0.13757 | **1.9003** | **1.72E-02** | 2.0298 | 7.01E-02 |

\* unknown; \*\* 4-Methyl-N-(1,2,3,4-tetrahydro-isoquinolin-1-ylmethyl)-benzenesulfonamide

Table 8-SM. Results from the pathway analysis obtained from Metaboanalyst 5.0. All the presented pathways had an impact value >0.1, which was calculated from pathway topology analysis; the total number of compounds in the pathway is shown in brackets after the pathway name; “Hits” indicate the actually matched compounds from the pathway; -log(p) comes from the p-value calculated in the enrichment analysis.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **OFMSW** | | **PS** | | **SS** | |
| **Pathway Name** | **Hits** | **-log(p)** | **Hits** | **-log(p)** | **Hits** | **-log(p)** |
| Aminoacyl-tRNA biosynthesis (46) | L-Phenylalanine  Serine  Alanine  L-Isoleucine  Proline | 4.108 | \_ | \_ | \_ | \_ |
| Alanine, aspartate and glutamate metabolism (22) | Alanine  GABA  Fumaric acid | 2.8533 | GABA | 1.3578 | \_ | \_ |
| Tyrosine metabolism (16) | Dopamine  Fumaric acid | 1.921 | \_ | \_ | \_ | \_ |
| Isoquinoline alkaloid biosynthesis (6) | Dopamine | 1.1999 | Dopamine | 1.9173 | Dopamine | 1.7931 |
| Butanoate metabolism (17) | GABA | 0.77149 | GABA | 1.4683 | \_ | \_ |
| Phenylalanine metabolism (9) | L-Phenylalanine | 0.94754 | \_ | \_ | L-Phenylalanine | 1.5321 |
| Starch and sucrose metabolism (22) | Maltose | 0.67028 | \_ | \_ | \_ | \_ |
| Glycine, serine and threonine metabolism (33) | Serine | 0.51762 | \_ | \_ | \_ | \_ |

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**Table 10-SM.** Primer sequences of reference and stress genes in *L.sativa*.

**GENE PRIMERS (5’-3´)**

APT1 forward: CTGTACAAGAAGGAGAACGAGC

reverse: ACGAGCACATACAGTGGCTT

EIF2A forward: GTTAAGAATGACCTGGCGAAACTGC

reverse: AAGGTGGGATTTCAAGAGTAAGACCC

EIF4A1 forward: CTTCATAGGATTGGGCGAA

reverse: TATGAGATCCGCAACATTGG

TIP41 forward: GCTGGAGGGAAACTACATTTAAGGC

reverse: CCACAGTTAACAGGGTATCAGGAAGG

PIP2 forward: CACTTCCCATCGGTTTCGCTG

reverse: CCGATGAATGGTCCCACCCAG

PAL forward: GGAGAGACGCTTACAGTTTCTCAG

reverse: TTGCTTAGTTCTCCGGTGAGAG

MIPS1 forward: AAGAGCCTTCTCCCTATGGTGAACC

reverse: CAGGGAGTGGGACCATGGATTC

P5CS forward: CGGTTTGGTCTTGGTGCAGAGG

reverse: GGTGGTGGTGGTGGTGAGGTC

HPPD forward: GCCCGGGTTTGAACCAGTTGAAAAG

reverse: CCGGCGCCTTCGTTGTGTTCCAGATAC

-TMT forward: AGTGAGTTGGCTCGAGTGGCTG

reverse: CAGCGGTAGAACACCAAGCAGG

L-GalDH forward: CCTCTCGGCAGCGTCTTCG

reverse: CCTCCCGCACTTTGTTGATACG

CAT forward: CACAAAGACATCGCCTTGGACC

reverse: TGTTCAGCATGGCGAGCAGG

SOD forward: TAAGTTCAATGGCGGAGGTCATGTG

reverse: TTCAGCATTCATCTTGGCAATCAAC

HSP70 forward: GGTTGTTGGAATCGATCTGGGAATC

reverse: ATCTGTCCCACCAACCTATCTCCG

MT2A forward: TGTTCAGGTAACTGCGGTTGTGG

reverse: TCTCTGATCCATCGTGACAACTCTTG

DREB2A forward: ATTATGGCTTGGAACTTTCGGGTCAG

reverse: TGAAGCACCATTAGCAACCACCATTG

WRKY70 forward: TGATCGCAGAGCTGATTAAAGGCAG

reverse: TCCGCAAGAACTCAACACCGAAAG

**Table 11-SM.** pH and electrical conductivity of the soils fertilizedwith either chemical fertilizer (CF), organic fraction from municipal solid waste ( OFMSW), sewage sludge (SS) or pig slurry (PS).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | pH | Electrical conductivity (dS/m) | Na (mg/kg dw) | Mg(mg/kg dw) |
| CF | 7.0±0.1a | 0.27±0.09a | 117±8a | 426±22a |
| PS | 7.0±0.4a | 0.29±0.03a | 121±5a | 440±18a |
| OFMSW | 7.2±0.1a | 0.26±0.04a | 121±4a | 445±16a |
| SS | 7.1±0.1a | 0.27±0.11a | 125±5a | 416±20a |

\* the values with different superscript letters in a column are significantly different (p<0.05)

PS

CF

OFMSW

SScv



**Fig 1.** pictures of the plots showing radish and lettuce vegetables fertilized with either chemical fertilizer (CF), organic fraction from municipal solid waste ( OFMSW), sewage sludge (SS) or pig slurry (PS).