High resolution HPLC-MS confirms overestimation of urea in soil by the diacetyl monoxime (DAM) colorimetric method

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Soil Biology and Biochemistry

DOI:
10.1016/j.soilbio.2019.04.015

Published: 01/08/2019

Peer reviewed version

Table 1 Concentration of reference compounds and responses for initial cross-reactivity tests of the DAM colorimetric method. N means no response was observed at the tested concentration.

Compound	**Concentration (mg l^{-1})**	**Response (mg N l^{-1})**
Allantoin	2.11	0.110
Alloxan	2.04	0.120
Amino acid mix	2.4	N
Amino sugar mix	3.1	N
Biuret	2.04	0.05
Fructose	2.0	0.01
Glucose	2.0	N
Hydantoin	2.02	0.190
Hypoxanthine	2.02	0.080
Myo-inositol	2.0	N
Nucleotide mix	3.9	0.111
Ribose	2.1	N
Sucrose	2.1	0.020
Xanthine	2.01	0.213

Table 2 Relative response of cross-reacting compounds to urea under the DAM colorimetric conditions

Compound	**Response relative to urea**	
	0.1 mg N l^{-1}	**1.0 mg N l^{-1}**
Allantoin	13.25 %	7.5 %
Alloxan	8.7 %	3.6 %
Biuret	2.8 %	0.47 %
Hydantoin	1.4 %	2.1 %

Table 3 Determined urea concentrations in grassland soils determined by the DAM colorimetric method and the reference LC-MS assay. Further site information is available in Table S1.

Site	DAM Colorimetric method		LC-MS Reference Assay		Fold over-estimation
	Urea concentration μg g^{-1} of soil	S.D.	Urea concentration μg g^{-1} of soil	S.D.	
Royal Fort Gardens, Bristol	10.01	0.06	0.325	0.006	30.8
North Wyke, Okehampton, Devon	12.54	0.04	0.71	0.05	17.7
Merddwr, Conwy	2.25	0.04	0.097	0.007	23.3
West Harptree, Bristol	1.5	0.05	0.068	0.001	21.8
Morland, Penrith	3.2	0.5	0.441	0.004	7.3
Llawhaden, Narbeth	7.9	0.3	0.133	0.001	59.0
Briantspuddle, Dorchester	16.9	0.2	0.613	0.006	27.5
Sherborne, Cheltenham	6.65	0.09	0.379	0.004	17.5
Sampford Courtenay, Okehampton, Devon	17.53	0.08	0.510	0.005	34.4
Ubley, Bristol	1.72	0.05	0.162	0.002	10.6
Somerset Levels	1.69	0.2	0.114	0.001	14.8