

Supplementary information

Biochar and Cd Alter the Degradation and Transport of Kasugamycin in Soil and Spinach

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Methods for Supplementary data

Plant total Cd concentration: A 0.50 g plant sample was placed in a 100 mL beaker and predigested overnight, at room temperature, in a 10 mL solution of concentrated HNO₃ and HClO₄ (4:1, v:v). The following day, the beakers were placed on an electric heating plate, the temperature raised from 100 to 200 °C over 30 min, and then the temperature was increased to 250 °C until the solution changed to colorless and ~ 2 mL of solution remained. The solutions were then removed, cooled, brought to a 25 mL final volume, and filtered through a 0.45 µm membrane filter. The Cd concentration in the digestate was determined with Atomic Absorption Spectrophotometry (AAS, TAS-986, Persee, China).

Soil available Cd concentration: A 5.000 g soil sample was mixed with 10 mL of 0.005 mol L⁻¹ DTPA (pH=7.3) in 50 ml centrifuge tube, by shaking at 180 r min⁻¹ for 2 h at room temperature. Afterwards, the tubes were centrifuged to separate the liquid

from solid phase, and liquid was filtered through a 0.45 μm membrane filter. The Cd concentration in the filtrate was determined with Atomic Absorption Spectrophotometry (AAS) (TAS-986, Persee, China).

X-ray Photoelectron Spectroscopy (XPS). XPS was used to analyze surface properties in approximately 10 nm depth of biochars. XPS measurements were conducted using an ESCALAB 250Xi (THERMO FISHER, USA), equipped with a focused monochromatized Al K α radiation ($h\nu = 1486.6$ eV). The X-ray spot size was 500 μm . Survey scan spectra in the 1351 – 0 eV binding energy range were recorded with a pass energy of 100.0 eV, others in 20.0 eV.

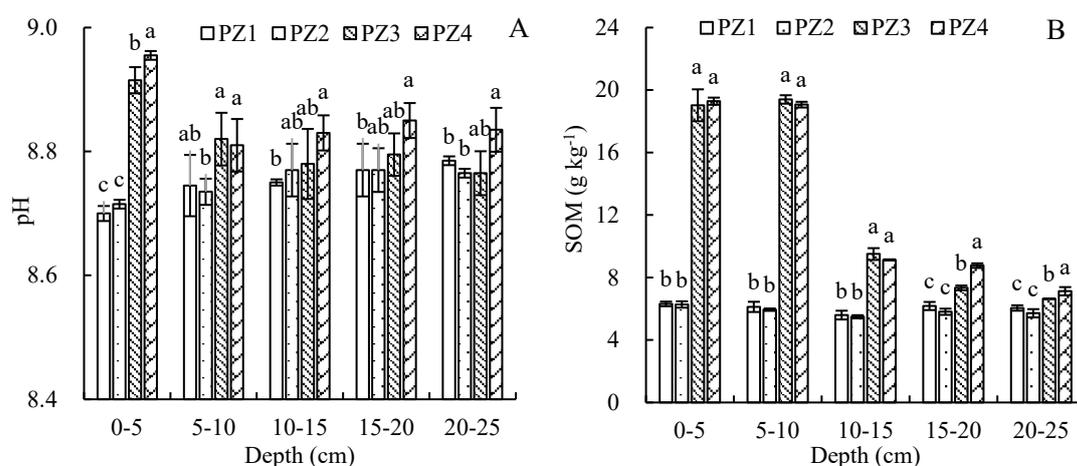
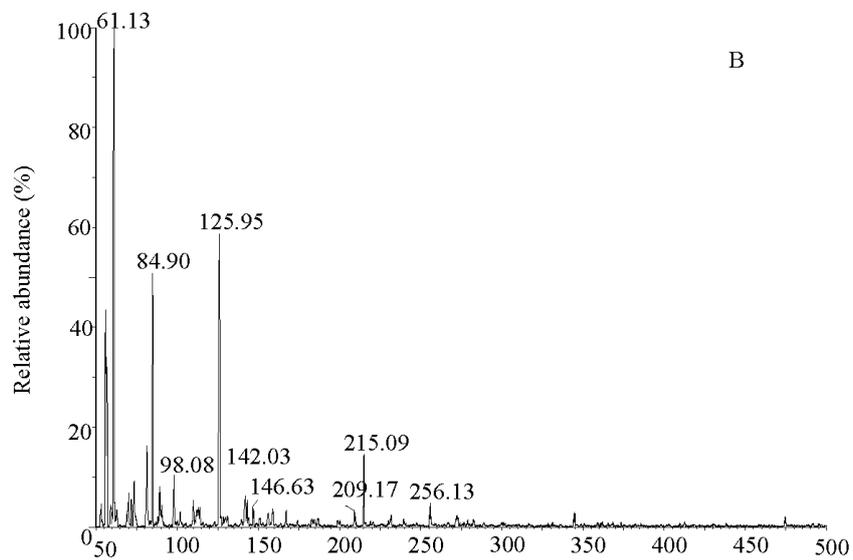
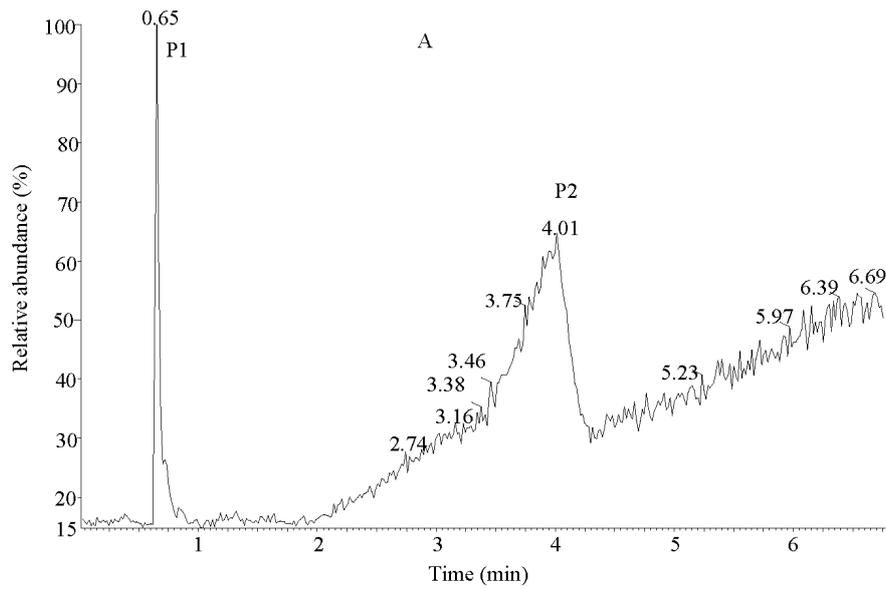


Figure S1. Changes in soil A) pH and B) soil organic matter (SOM) as a function of depth at the end of the study (28 days after KSM application). Treatment PZ1 = 10 mg KSM m⁻² was sprayed on spinach leaves in unamended soil; PZ2 = 20 mg Cd kg⁻¹ containing soil added to an unamended soil surface, and 10 mg KSM m⁻² sprayed on spinach leaves; PZ3 = 5% biochar amended soil (wt:wt), and 10 mg KSM m⁻² sprayed on spinach leaves; and PZ4 = 20 mg Cd kg⁻¹ containing soil added to the surface of a 5% biochar amended soil (wt:wt), and 10 mg KSM m⁻² sprayed on spinach leaves.

Different lower-case letters above error bars (error bars: the standard deviation of the mean; $n = 3$; $p < 0.05$, determined via a Tukey post-hoc test) indicate statistically significant differences between treatments within a specific soil depth.



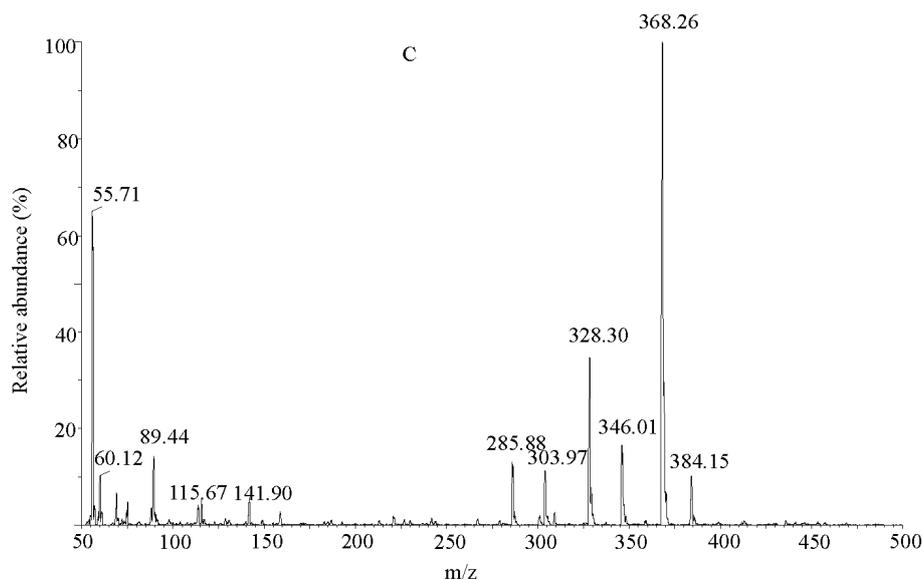


Figure S2. Chromatogram of soil KSM and degradation products after 1 day following treatment (PZ4; 20 mg Cd kg⁻¹ containing soil added to the surface of a 5% biochar amended soil (wt:wt), and 10 mg KSM m⁻² sprayed on spinach leaves) full-scan mass spectra(A: full-scan mass spectra; B: the peak P1 of A; C: the peak P2 of A).

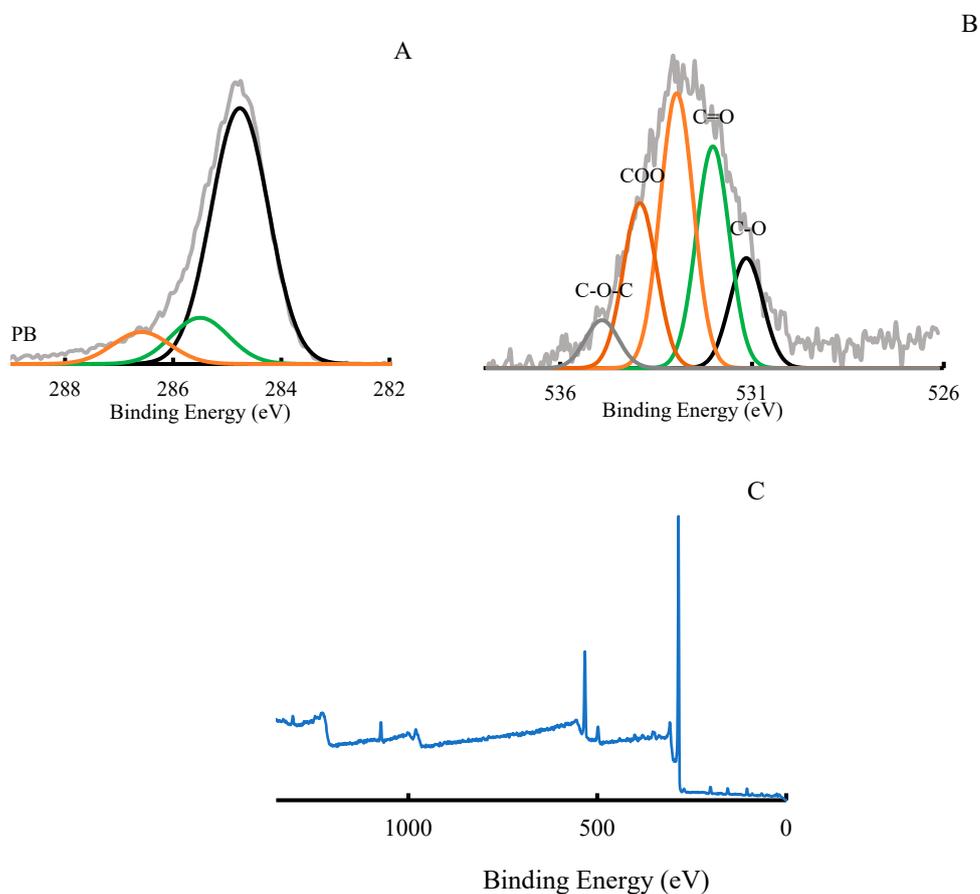
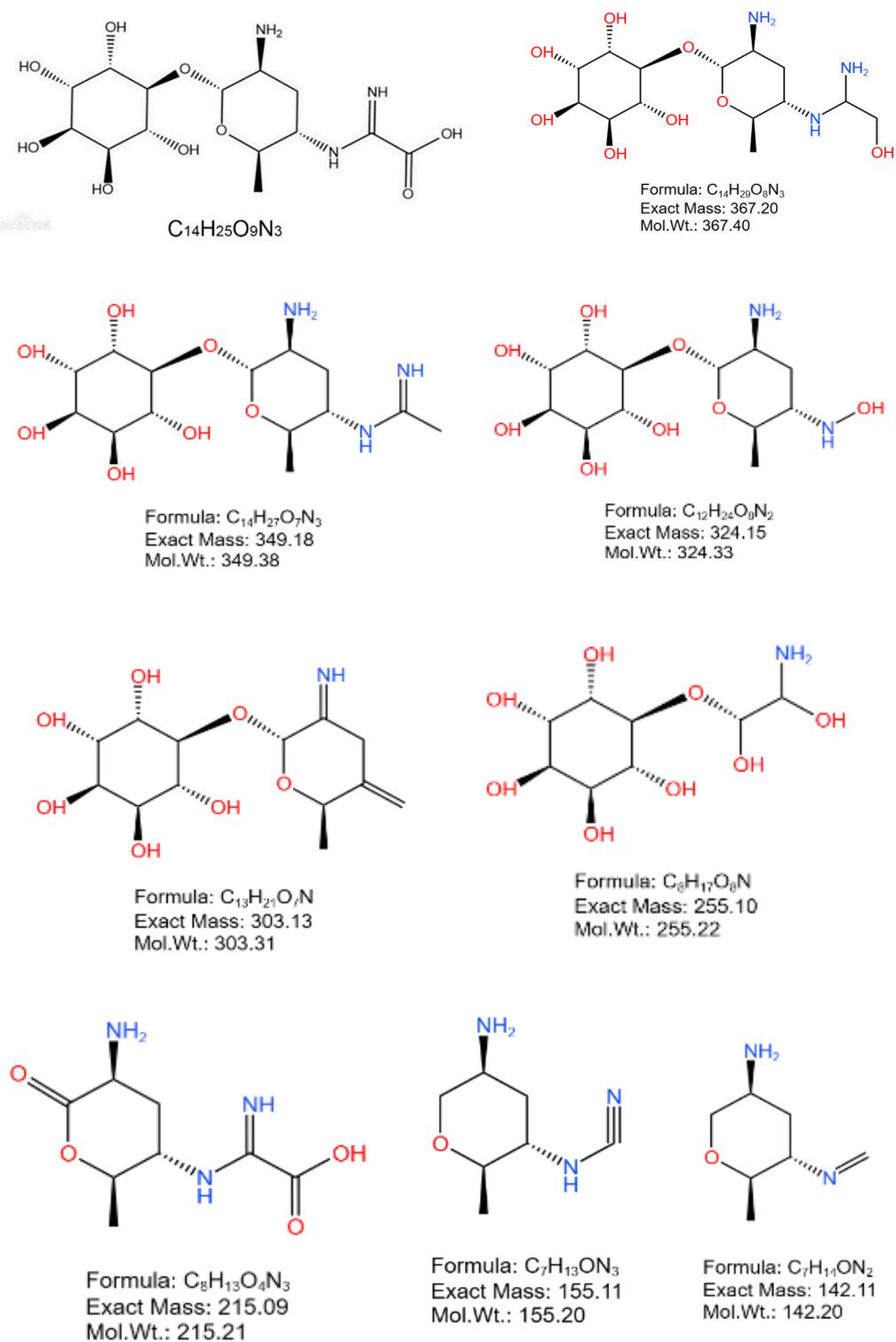


Figure S3 XPS peak fits of C1s (A), O1s and (B) and maps (C) from biochar.



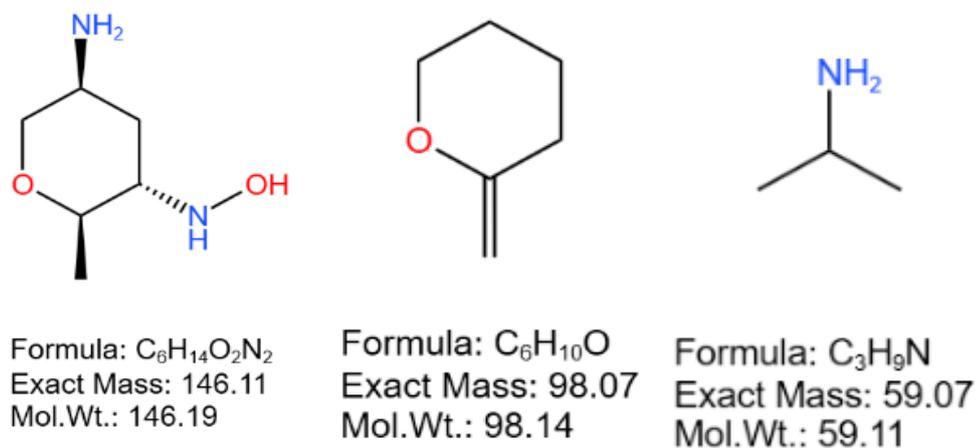


Figure S4. Possible KSM degradation products.

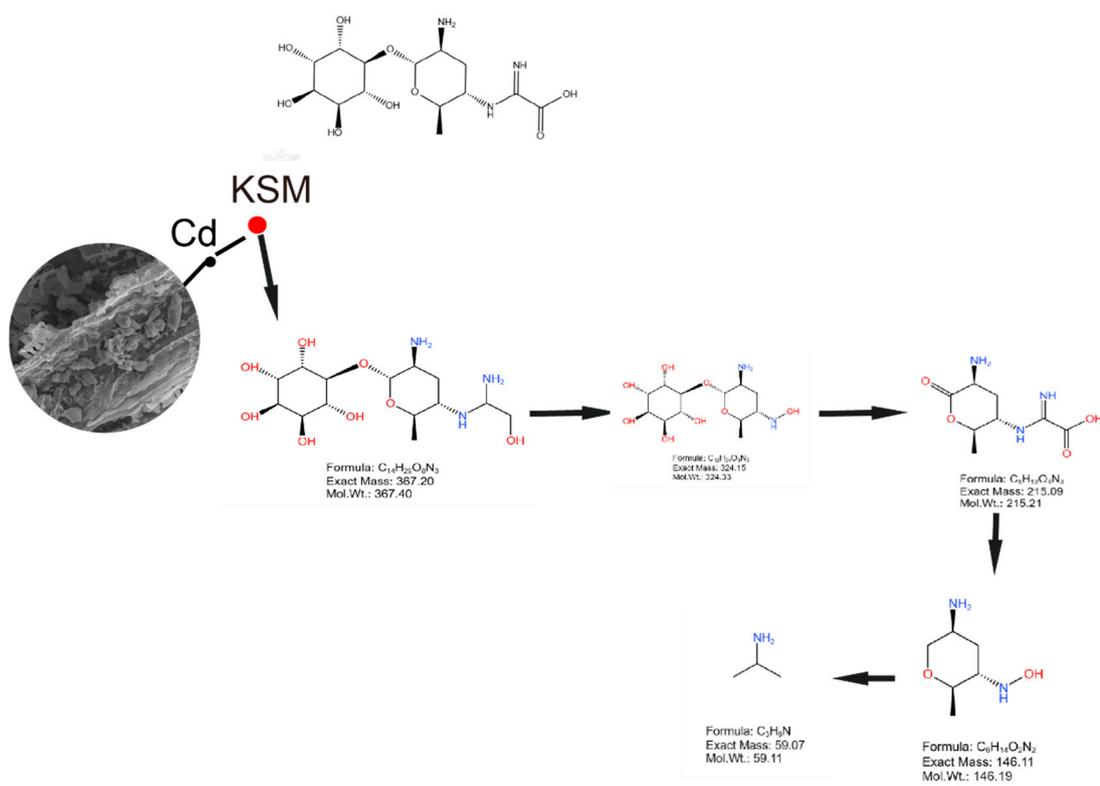


Figure S5 Possible KSM degradation routes.

Table S1. Total mean Cd concentration in spinach leaves and roots ($mg\ kg^{-1} \pm$ standard deviation of the mean). Treatment PZ2 did not receive biochar, while treatment PZ4 received 5% biochar application to soil. $n = 3$; $p < 0.05$, determined via a Tukey post-hoc test.

Treatment	Spinach leaf	Spinach root
PZ2	30.75 ± 0.31a	37.85 ± 0.05a
PZ4	25.47 ± 1.75b	35.80 ± 0.13b

Table S2. Soil bioavailable Cd concentrations with depth (DTPA, mg kg⁻¹ ± standard deviation of the mean). Treatment PZ2 did not receive biochar, while treatment PZ4 received 5% biochar application to soil. n = 3; p < 0.05, determined via a Tukey post-hoc test.

Depth (cm)	0-5	5-10	10-15	15-20	20-25
PZ2	1.00 ± 0.13a	0.54 ± 0.04a	0.14 ± 0.02a	0.06 ± 0.01a	0.05 ± 0.01a
PZ4	0.90 ± 0.07a	0.28 ± 0.03b	0.01 ± 0.01b	0.05 ± 0.01a	0.01 ± 0.00b

Table S3. Band assignments in FTIR spectra of biochar.

Bands (cm ⁻¹)	Assignments
3400-3320	-OH stretching
3000-2800	Aliphatic CH stretching
1630-1700	Aromatic carbonyl/carboxyl C=O/C=C stretching
1430-1420	Aromatic C=C stretching
1000-1157	C-O-C
840-880	Glucoside CH ₂ deformation
750-820	Aromatic rings
460-470	Si-O

Table S4. Band assignments in:

	Different time pH	Different depth pH	Different time SOC	Different depth SOC	Root biomass	Shoot biomass	Different time Soil KSM	Plant root KSM	Plant shoot KSM	Different soil depth KSM	Total Cd
Different time pH	1.000	0.597**	.827**	0.363	0.833	0.961*	-0.631	-0.907	-0.988	-0.828	-0.287
Different depth pH	0.597**	1.000	0.429	0.665**	0.766	0.801	-0.496	-0.910	-0.685	-0.573	-0.444
Different time SOC	0.827**	0.429	1.000	0.252	-0.674	-0.788	-0.299	0.617	0.890	-0.732	0.523
Different depth SOC	0.363	0.665**	0.252	1.000	-0.372	-0.595	-0.577	0.537	0.729	-0.349	0.856**
Root biomass	0.833	0.766	-0.674	-0.372	1.000	0.947	-0.775	-0.798	-0.900	-0.674	-0.660
Shoot biomass	0.961*	0.801	-0.788	-0.595	0.947	1.000	-0.926	-0.925	-0.982	-0.859	-0.826
Different time Soil KSM	-0.631	-0.496	-0.299	-0.577	-0.775	-0.926	1.000	0.874	0.972*	0.412	0.011
Plant root KSM	-0.907	-0.910	0.617	0.537	-0.798	-0.925	0.874	1.000	0.882	0.979*	0.735
Plant shoot KSM	-0.988	-0.685	0.890	0.729	-0.900	-0.982	0.972*	0.882	1.000	0.837	0.915
Different soil depth KSM	-0.828	-0.573	-0.732	-0.349	-0.674	-0.859	0.412	0.979*	0.837	1.000	0.702*
Total Cd	-0.287	-0.444	0.523	0.856**	-0.660	-0.826	0.011	0.735	0.915	0.702*	1.000

** The correlation is significant at a $p < 0.01$.

* The correlation is significant at a $p < 0.05$.