SUPPORTING INFORMATION

Monitoring of CO as a plant signaling molecule under heavy metal stress using carbon nanodots

Shrodha Mondal,[a] Olivia Sarkar,[b] Santi M. Mandal, [c] Ansuman Chattopadhyay,[b] and Prithidipa Sahoo*[a]

[a]Department of Chemistry, Visva-Bharati University, Santiniketan-731235, India

[b]Department of Zoology, Visva-Bharati University, Santiniketan-731235, India

[c]Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur-721302, India

∗Correspondence to: Prithidipa Sahoo (Email: prithidipa.sahoo@visva-bharati.ac.in)

Number of Pages: 10

Number of Tables: 3

Number of Equations: 0

Number of Figures: 9

Table S1. Performance comparison of different methods of CO detection.

Methods	Material applied	Linear Range	Limit of Detection	Application	References
Fluorometry	carbonic oxide Probe	0-10 µM	0.38 µM	In situ imaging of CO in Arabidopsis thaliana plant tissues	[1]
Fluorometry	fluorescent probe based on Cu^{2+} modulated polydihydroxyphenylalanine nanoparticles (PDOAs)	-	72.4 nM	Tracking of CO in plant tissues	[2]
Fluorometry	hemicyanine-based off-on fluorescent probe, CO-H1	-	0.048 µM	Monitoring endogenous mitochondrial CO levels in living cells	[3]
Two-photon fluorescent probe based on carbazole-coumarin fused ring	Cyclometallated Pd complex	-	0.653 µM	Imaging carbon monoxide in living tissues	[4]
Fluorometry	palladium-mediated carbonylation	500 nM-10 µM	1 µM	Selective imaging of carbon monoxide in living cells	[5]
Fluorometry	Nitrogen-doped Carbon Quantum Dots (N-CQDs)	2.43-47.51 µM	0.102 µM	Detection and monitoring of carbon monoxide (CO) activity in plant tissues	**This Work**

1. Synthesis of N-CQDs

A solution was prepared by combining 3-aminophenol (0.2 g) and diethylenetriamine (1 mL) with 40 mL of double-distilled water in a 100 mL Erlenmeyer flask. The mixture underwent continuous stirring for 4 to 6 hours before being exposed to microwave irradiation at 560 W for 4 minutes (Scheme 1). The solution was centrifuged for 15 minutes at 10,000 rpm after cooling, followed by filtration through a 0.22 µm membrane filter. The N-CQDs solution was stored at 4°C for future applications and studies.

2. Characterization

The morphology of the N-CQDs was analyzed using an HR-TEM JEOL JEM 2100 transmission electron microscope. DLS data were recorded using a Malvern 4800 Autosizer equipped with a 7132 correlator. FT-IR spectra were obtained using a Nexus TM 870 spectrophotometer. XPS data were collected with the PHI

5000 VERSA PROBE III ULVAC from PHI (Physical Electronics), USA. EDX analysis was performed at the ZEISS Scanning Electron Microscope Laboratory. Fluorescence spectra were precisely recorded on a Hitachi Model F-7100 Fluorescence Spectrophotometer, and UV absorption spectra were acquired using a Hitachi U-2910 spectrophotometer. Time-correlated single-photon counting (TCSPC) fluorescence measurements were conducted with an advanced multifunctional spectrophotometer (Model 1057, Fluorlog, Horiba Scientific Tech., USA). Centrifugation was done using the Benchmark-Hermle Z216 MK Refrigerated Benchtop Centrifuge. The confocal imaging study was conducted using a Leica TCS SP8 laser scanning confocal microscope.

Determination of Quantum Yield (QY):

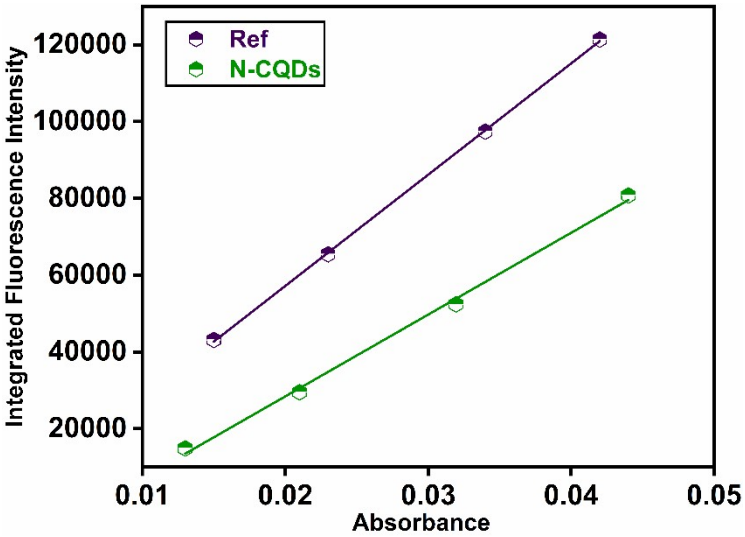

Figure S1. Calibration curves of integrated fluorescence intensity (area) against absorbance for Reference (Green) and N-CQDs (Purple). From the plot we get the slop of reference = 2.89898×10^6 and slop of the sample (N-CQDs) = 2.13142×10^6.

Sl. No.	UV-vis absorbance	Area of FL spectra (Sample)	UV-vis absorbance	Area of FL spectra (Reference)
1.	0.013	14857.0619	0.015	43125.00
2.	0.021	29496.1788	0.023	70691.00
3.	0.0319	52388.9046	0.034	97399.00
4.	0.044	80750.0707	0.042	121362.00

QY of reference is 0.54

Slope of Reference (Standard) is 2.89898×10^6

Slope of sample is 2.13142×10^6

Refractive index is same for both samples.

So, the QY of the sample is = $(0.54\times2.13142\times10^6)/ (2.89898\times10^6) =0.39 = 39\%$

FT-IR spectra of (N-CQDs+CO) complex:

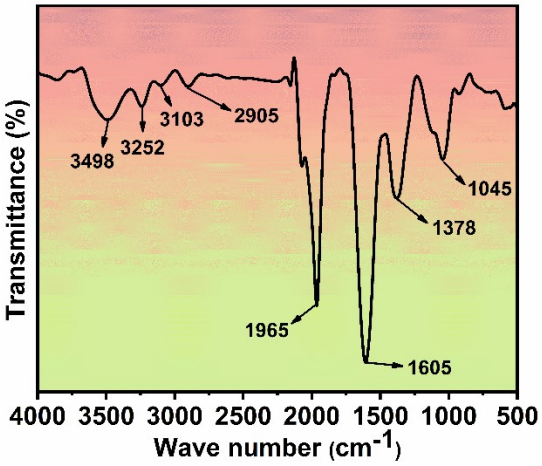

Figure. S2 FT-IR spectra of (N-CQDs+CO) complex.

High-resolution XPS spectra of N-CQDs:

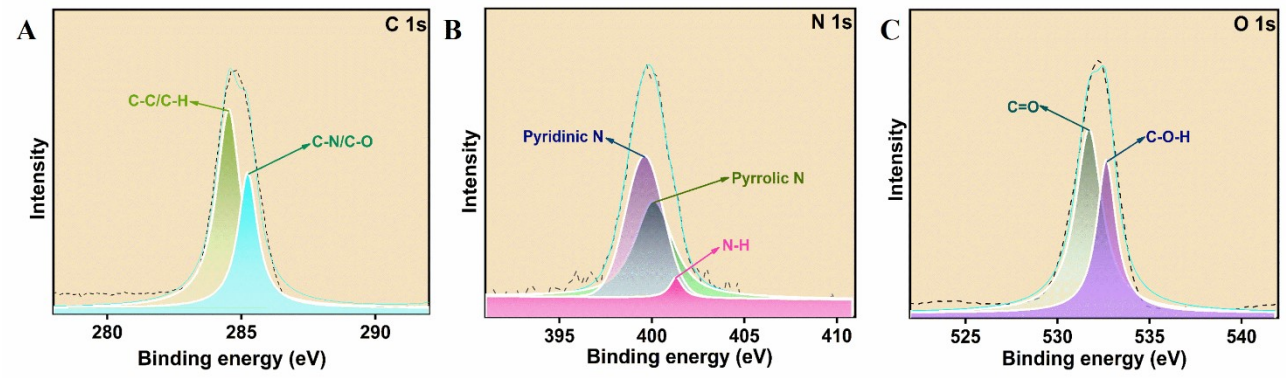

Figure. S3 High-resolution XPS spectra of N-CQDs (A) C 1s, (B) N 1s, and (C) O 1s.

UV-Titration:

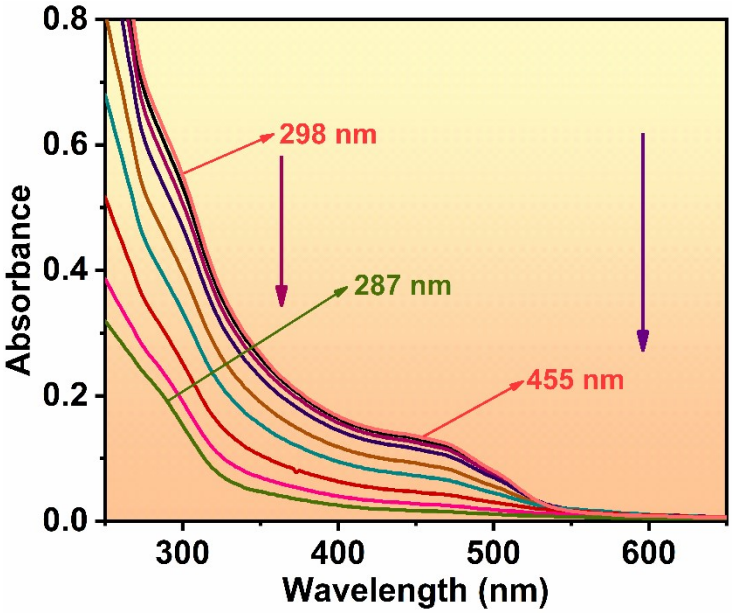

Figure. S4 UV-vis absorption spectra of N-CQDs upon addition of CO (10^{-4}M).

Binding constant calculation graph (Fluorescence method):

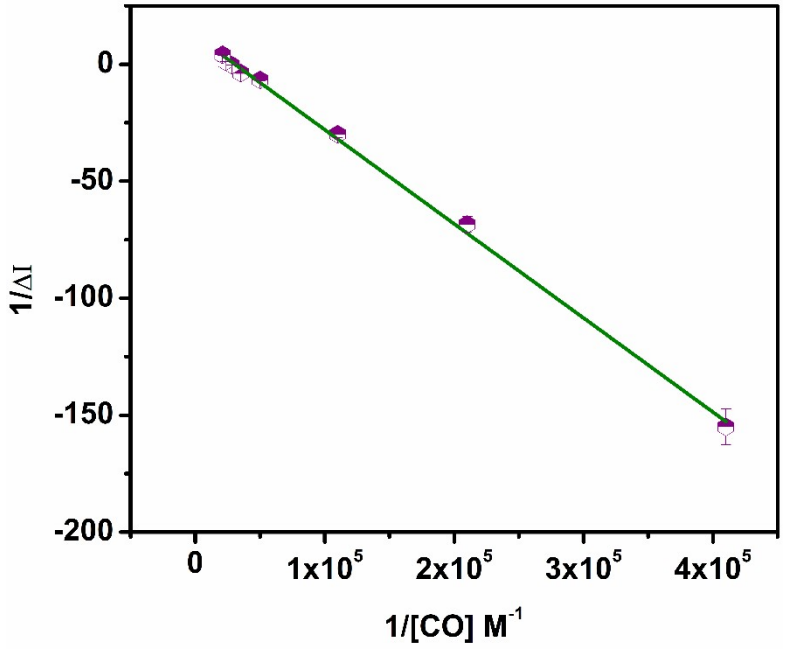

Figure. S5 Linear regression analysis for the calculation of association constant value by fluorescence titration method.

The association const. (K_a) of N-CQDs for sensing CO was determined from the equation: K_a = intercept/slope. From the linear fit graph, we get intercept=12.29513, slope =4.02433×10^{-4}. Thus, we get $\mathbf{K_a}$= (12.29513) / (4.02433×10^{-4}) = $\mathbf{3.055 \times 10^4\ M^{-1}}$

Table S2. Calculation of Standard Deviation and Limit of Detection (LOD) for CO

Blank Reading (N-CQDs)	**Fluorescence Intensities at 389 nm (X)**	**Mean (X)**	**Standard Deviation (σ) = $\sqrt{\frac{\sum\|X-x\|^2}{N}}$**
Reading 1	781.3	780.84	0.441
Reading 2	781		
Reading 3	781		
Reading 4	780		
Reading 5	780.9		

Slope, m for CO = 1.29307×10^7
LOD for CO= 3σ/m = $(3 \times 0.441) / (1.29307 \times 10^7) = 0.102 \times 10^{-6}$ M = 0.102μM

Determination of limit of quantification (LOQ):

The limit of quantification (LOQ) = 10σ/m = $(10 \times 0.441)/ (1.29307 \times 10^7)$ = 0.341 μM

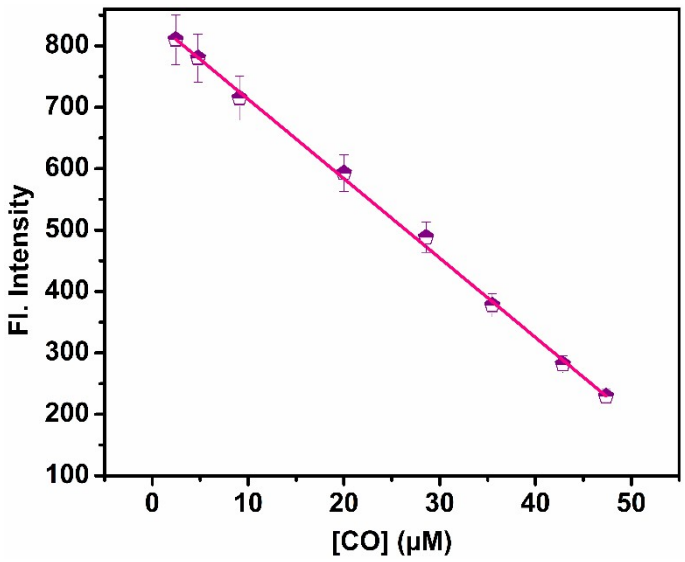

Figure. S6 Linear fit curve of N-CQDs at 389 nm with respect to CO concentration.

Selectivity:

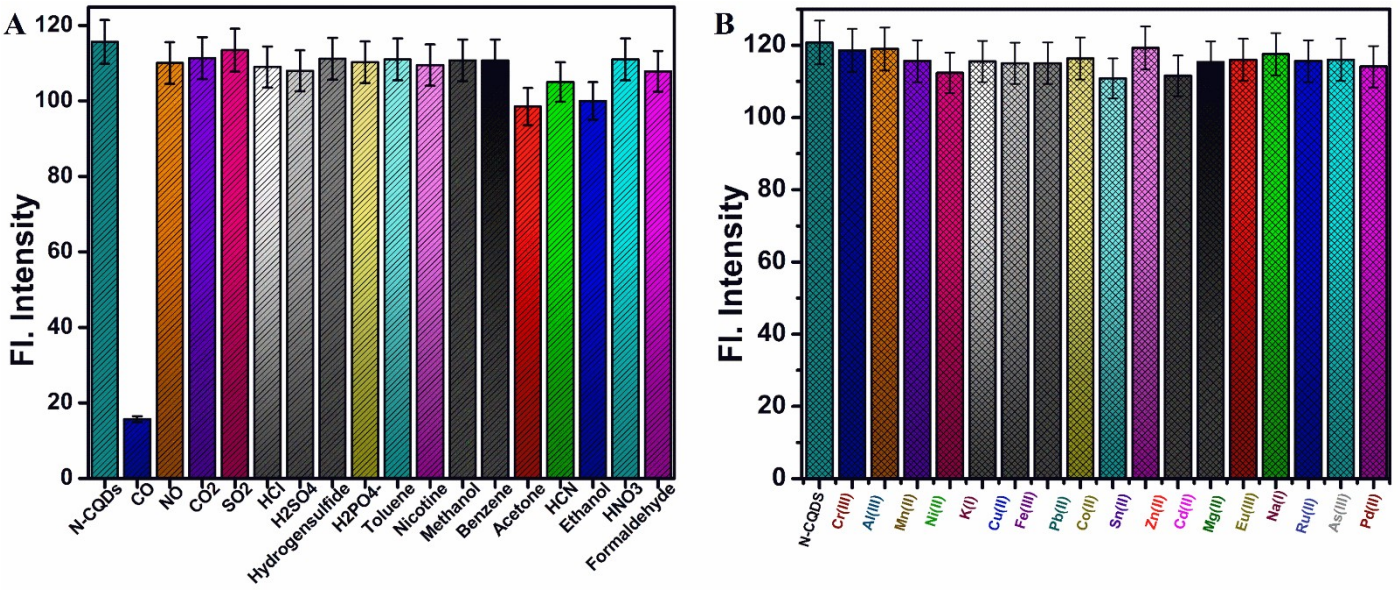

Figure. S7 Competitive fluorescence spectra of N-CQDs with different analytes and metals at 389 nm (λex = 320 nm).

Fluorescence lifetime decay:

Table S3. Decay time components of N-CQDs, N-CQDs + CO

System	b_1	τ_1	b_2	τ_2	$<\tau>=b_1\tau_1+b_2\tau_2$
N-CQDs	0.072904694	10.7864	0.927095305	0.7826692	1.51 ns
N-CQDs + CO	0.087093379	11.0641	0.91290662	0.816404	1.71 ns

Confocal microscopic images of transverse sections of seed of black chickpeas and mung beans:

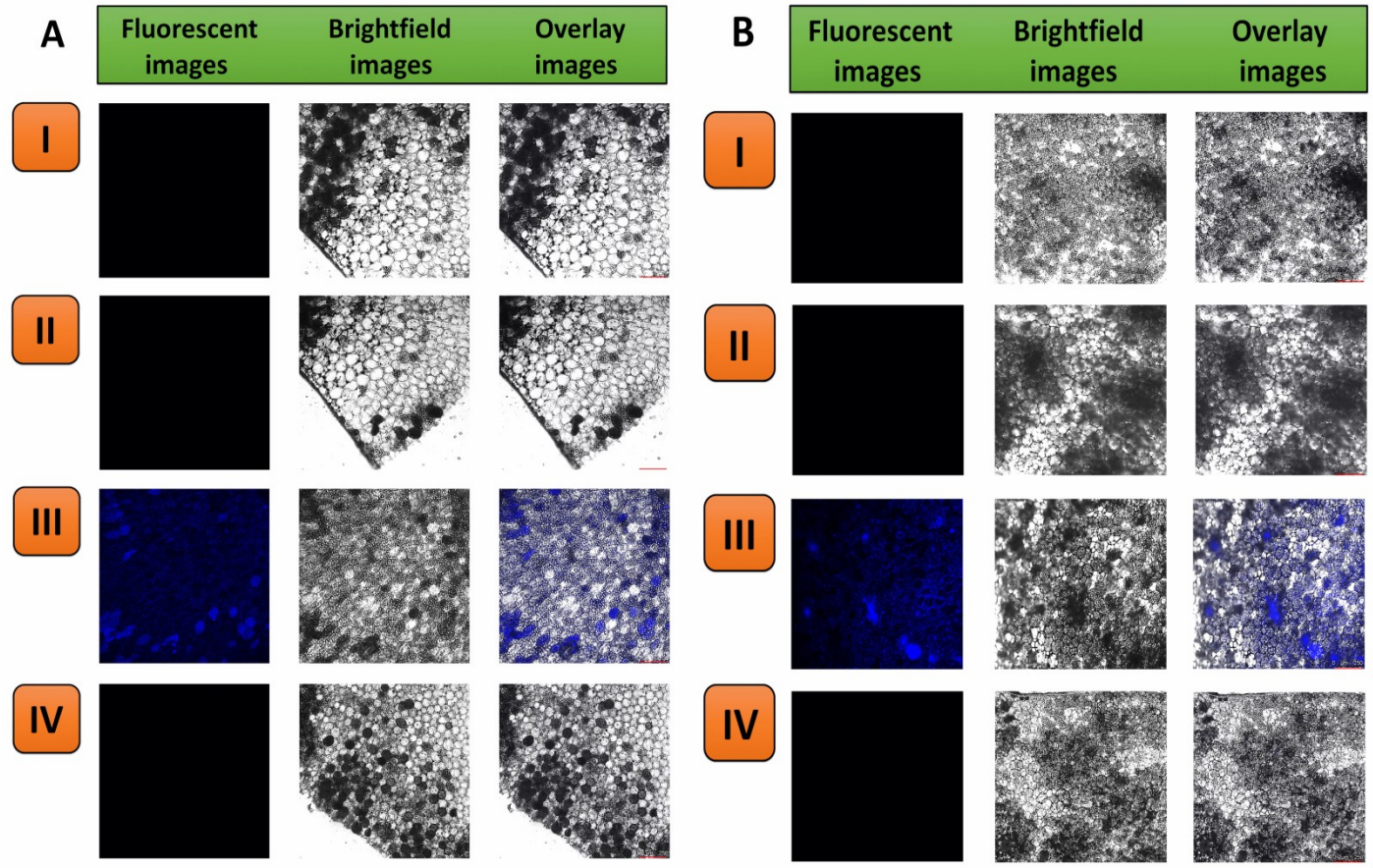

Figure. S8 Confocal microscopic images of transverse sections of seeds of (A) black chickpeas (*Cicer arietinum*) and (B) mung bean (*Vignaradiata*) after treatment with quantum dot (N-CQDs), carbon monoxide (CO), and heavy metal. (AI-BI) control seed section; (AII-BII) seed section treated with 10^{-3} M of heavy metal; (AIII-BIII) seed section treated with 10^{-3} M of N-CQDs following prior exposure to heavy metals and (AIV-BIV) seed section pre-treated with CO (10^{-3} M) after treatment with heavy metal and N-CQDs. The left, middle, and right panels display fluorescent, brightfield, and overlay images respectively. Magnification: 100X. Zoom factor: 0.75. Scale bars: 0-250 µm.

Densitometric analysis:

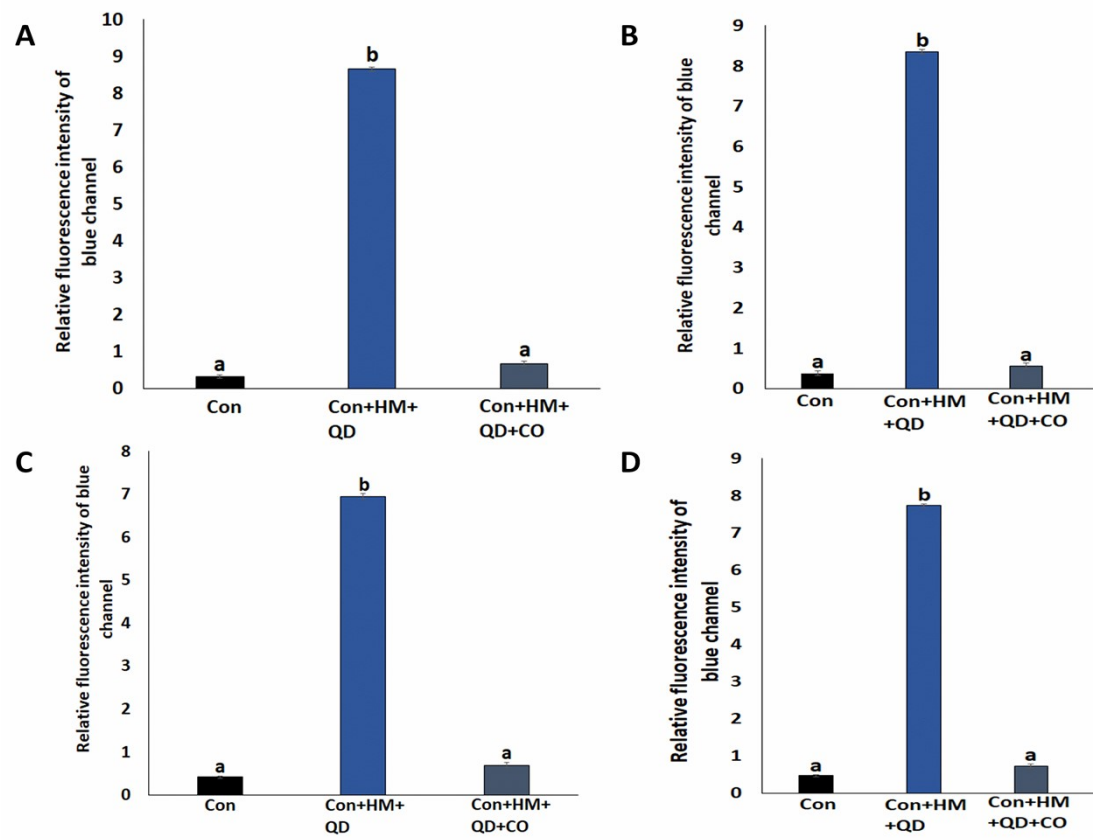

Figure. S9 Densitometric analysis of fluorescence intensity was quantified from the confocal microscopic images of untreated and treated root sections of A) black chickpea plants, seed sections of B) black chickpea plants, root sections of C) mung bean plants, and seed sections of D) mung bean plants by using ImageJ v 1.46 software and graphed. Values are expressed as mean ± SEM. Means with different letters are significantly different. Significance level $\alpha = 0.05$. [(Con: control, Con+HM+QD: control, heavy metal and quantum dot, Con+HM+QD+CO: control, heavy metal, quantum dot and carbon monoxide.]

REFERENCES

1. Y. Cao, Y. Xu, N. Fang, Q. Jiao, H. L. Zhu, Z. Li, *Plant Physiol*., 2023, **193(2)**, 1597-1604.
2. X. Mu, Y. Wang, J. Xu, F. Zeng, *Anal Methods*., 2024, **16(36)**, 6201-6209.
3. M. Yi, N. Zhang, X. Liu, J. Liu, X. Zhang, Y. Wei, D. Shangguan, *Spectrochim Acta A Mol Biomol Spectrosc*., 2023, **291**, 122377.
4. K. Zheng, W. Lin, L. Tan, H. Chen, H. Cui, *Chem. Sci*., 2014, **5 (9)**, 3439-3448.
5. B. W. Michel, A. R. Lippert, C. J. Chang, *J Am Chem Soc*., 2012, **134(38)**, 15668-71.