Supplementary Information (SI) for Nanoscale.

Enhanced Absorption and Fluorescence quenching methods for the quantitative analysis of Cr(VI) ions using avocado seed-derived carbon quantum dots as pseudo-derivatizing reagents

Amahle Mkhize[1], Xolani Nocanda[2], Irvin Noel Booysen[1], Allen Mambanda[1]

[1]School of Chemistry and Physics, College of Agriculture and Science, University of KwaZulu-Natal, Pietermaritzburg, 3201

[2] Council for Scientific and Industrial Research, Water Centre, Brummeria, Pretoria, 0001, South Africa

SI 1. Avocado Seed Powder Preparation.

Five avocado fruits were cut open into halves, the seeds were removed, and the brown protective sheet was peeled off. The seeds were rinsed with small amounts of ultra-pure water, ethanol, and acetone (5 mL). The seeds were cut into small pieces and air-dried for a week. These were ground into powder using a Warring blender and dried in an oven for 7 h at 70 °C. The brown-orange powder was weighed, stored in a polyethylene container, and kept in a desiccator until analysis.

S1 2. Determination of quantum yield (Q_Y) of the S, N-CDs

The photoluminescence quantum yield (Q_Y) of the avocado seed-derived S, N-CDs was determined by measuring the absorption spectra and PL intensity of the fluorescence (FLUO) and the absorption along with that of the 0.1 M quinidine sulfate dihydrate (reference material) in *ca*. 0.05 M sulfuric acid. Q_Y is calculated using equation 1:

$$Q_Y = Q_R \times (I_S/I_R) \times (A_R/A_S) \times (\eta^2{}_S/\eta^2{}_R) \text{……………… Eqn. 1,}$$

where Q_R is the known quantum yield of reference/standard solution (0.1 M quinine sulphate in sulfuric acid), which is 0.54 (54%), $I_{R,}$ A_R, $I_{S,}$ and A_S are the fluorescence intensities and absorbances of the reference and the S, N-CDs, respectively, and $\eta_{r/s}$ is the refractive index of the solvents. The excitation wavelengths of the quinine sulfate and S, N-CDs were 317 nm and 310 nm, respectively.

S1 3. Evaluation of the selectivity of S, N-CDs for Metal Ion Sensing

S1 4. Verifying the absorbance enhancement of the $Cr_2O_7^{2-}$ (Cr(VI)) ions in S,N-CDs solutions.

To verify the absorbance enhancement of the S, N-CDs with $Cr_2O_7^{2-}$, spectra of 200, 500, and 1000 ppb Cr(VI) in ultrapure water, along with those in S, N-CD solutions, were recorded and presented as overlays. The absorbances at 360 nm and 375 nm, respectively, were compared. The three Cr(VI) in S, N-CD standard solutions were prepared by mixing variable volumes of S, N-CDs with 20, 40, and 80 µL of 10 mg/L Cr(VI) standards to attain the final concentration of 200, 500, and 1000 ppb and a final volume of 2.00 mL.

S1 5. Metal ion detection/sensing by the S, N-CDs, and interference studies.

To test for the applicability of the synthesized S, N-CDs as a chemical sensing reagent for detecting heavy metals and anions, the absorbance or fluorescence (FL) /photoluminescence (PL) of the S, N-CDs in the absence and presence of trace levels of selected metal ions and anions were studied. Stock standard solutions (100 mg/L (ppm) of each test ions (of various eco-toxicity and oxidation states) were prepared by accurately weighing appropriate masses of $CdCl_2$ (for Cd(II)), $PbCl_2$ (for Pb(II)), $HgCl_2$ (Hg(II), As_2O_3 (for As(III)), $Al(NO_3)_3.9H_2O$ (for Al(III)), $NiCl_2$ (for Ni(II)), $CoCl_2$ (for Co(II)), $CrCl_3$ for Cr(III) (for $CuSO_4$ (for Cu(II), $FeCl_2$ (for Fe(II)), $MgSO_4$ (for Mg(II)), NaCl, (for Na(I)), K_2CO_3 (for K(I)), VCl_3 (for V(III)), $K_2Cr_2O_7$ (for Cr(VI)) and $KMnO_4$ (for Mn(VII)). These stocks were subsequently diluted to 50 mg/L. aliquots of 20 µL of the 50 mg/L ion standards were added to 1980 µL S, N-CDs (8.3 mg/mL). The final concentration of each metal ion in the S, N-CDs (final volume = 2.0 mL) was 0.50 mg/L (500 ppb). The S, N-CDs (without added ions) and metal-ion spiked S, N-CD CD mixtures were vortexed (20 s) before their absorption or emission spectra were recorded as overlays. The normalized absorbance or fluorescence data were analysed at 375 nm and 420 nm (after excitation with 310 nm radiation for PL). Marked spectral changes of the S, N-CDs were observed (as an increase in absorbance or quenched fluorescence emission) only for the Cr(VI) ions in the S, N-CD solution. Subsequently, further experiments were repeated to test the selectivity/specificity of the two modes of Cr(VI) detection in the presence of potential interfering ions as may occur in complex environmental samples. The absorption or fluorescence emission spectra of the 500 ppb Cr(VI) in S, N-CDs, and in the presence of an interferent ion at equal concentration of 500 ppb or 100-fold excess using a 100-ppb solution were recorded. This was done by adding 20 µL of each to the S, N-CDs, adjusting the final volume to 2.0 mL.

S1 6. Effect of pH on the enhanced absorption and fluorescence quenching of Cr(VI) in S, N-CDs solutions

To evaluate the optimum pH of the enhanced absorption and fluorescence quenching of the S, N-CD by the $Cr_2O_7^{2-}$ pH buffered solutions (in the range 2 to 12), 100 ppb Cr(VI) in S, N-CDs were prepared using the 0.1 M NH_4Cl/NH_3 buffer system of variable mole adjusted with 0.1 M HCl or 0.1 M NaOH. The normalized absorbance or fluorescence of the CDs and Cr(VI) spiked solutions was measured at 375 nm and 420 nm upon excitation with 310 nm radiation, respectively.

S1.7. Photostability, ionic interferences, and photo-oxidation of fluorescence (FLUO) of the S, N-CDs

To evaluate the photobleaching/stability of fluorescence (FLUO) emission of the S, N-CDs, an aliquot of S, N-FCDs at the optimum pH was irradiated with an 850 W lamp for up to 180 minutes, and the normalized FLUO was measured every 30 minutes at 420 nm upon excitation with 310 nm radiation. The stability of the S, N-CDs emission in the presence of 0.2 - 1.0 M NaCl (an electrolyte) or H_2O_2 oxidizing agent was evaluated. Aliquots of 20 µL of 4 M NaCl and 0.2 - 1.0 M H_2O_2 were added to the S, N-CDs, respectively. The normalized absorbance or fluorescence was measured at 375 nm and 420 nm (after excitation with 310 nm radiation).

SI.8. HGSIA and FLUO quenching determination of the Cr(VI) in the uMsunduzi river and a positive control water sample (laboratory wastewater containing the dichromate ion) by standard addition

A 20 µL (N, S CDs only) aliquot and variable (0 - 200 µL) aliquots of 10 mg/L Cr(VI) in S, N-CDs were spiked to 2.00 mL of laboratory wastewater contaminated with Cr(VI) ions (a positive control) to attain spiked concentration in the range of 0 - 1000 ppb. The enhanced absorption or quenched fluorescence of the unspiked water and spiked with Cr(VI) + S, N-CDs in the positive control samples were recorded at 375 nm and 420 nm (after excitation with 310 nm radiation), respectively. The Cr(VI) concentration in the laboratory waste sample was deduced from the standard addition calibration equation obtained from both HGSIA and fluorescence quenching.

SI 9. Evaluating the precision and accuracy of the HGSIA and the quenched FLUOR methods for detecting Cr(VI)

The accuracy (as % Cr(VI) recovery) of the HGSIA or quenched FLUOR method for Cr(VI) measurements was evaluated at two low concentration levels in spiked uMsunduzi River water, which had been collected in the Pietermaritzburg area (Latitude: -27°37’8.39” and longitude: 30°40’21.59”). The water sample was centrifuged at 12,000 rpm to remove large particles and filtered with a 0.22 µm membrane filter. The water was used to prepare 8.3 mg/L N, S CDs. Two aliquots, 1.98 and 1.80 mL of the CDs (in river water), were spiked with 20 µL and 200 µL 10 mg/L Cr(VI) in N S-CDs standards, respectively. The final Cr(VI) concentration in the two spiked river water samples was 20 ppb and 200 ppb. The enhanced absorbances or FLUOR of the unspiked (N, S-CDs in water), 20 ppb and 200 ppb Cr(VI) in N, S-CDs/River water samples were recorded at 375 nm. No absorbance changes were recorded for the water on CDs in the River water. The recovered [Cr(VI)]s at the two spiked levels obtained by substituting the respective normalized absorbances or quenched FLUOR at the two spike levels in the calibration equation were used to calculate the % recovery.

To evaluate the reproducibility precision, the enhanced absorbance or the quenched fluorescence of the 20 ppb Cr(VI) in S, N-CDs/uMsunduzi water was recorded several times (N=3) at 375 nm and 420 nm (after excitation with 310 nm radiation), respectively. The data was expressed as % relative standard deviation (%RSD).

SI 10. ICP-OES determination of the Cr(VI) in spiked River and positive control water samples.

The ICP-OES spectrometer was calibrated with eight serial diluted Cr(VI) in S, N-CDs standard (0.5 - 1500 ppb range) under the following instrumental settings: the concentrations of the spiked 20 ppb and 100 ppb uMsunduzi River water samples and the laboratory waste sample were determined from the calibration equation and the concentration values for all the samples were compared with those of the HGSIA and quenched FLOUR methods.

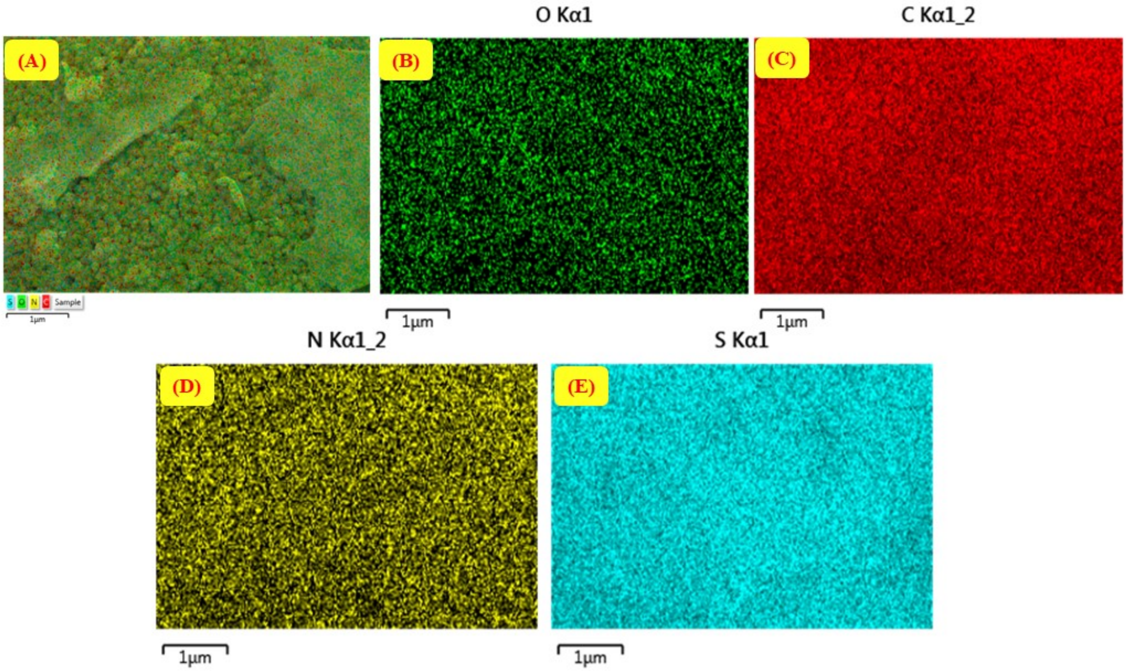

Figure S1. *SEM-EDX elemental maps of the S, N-FCDs*

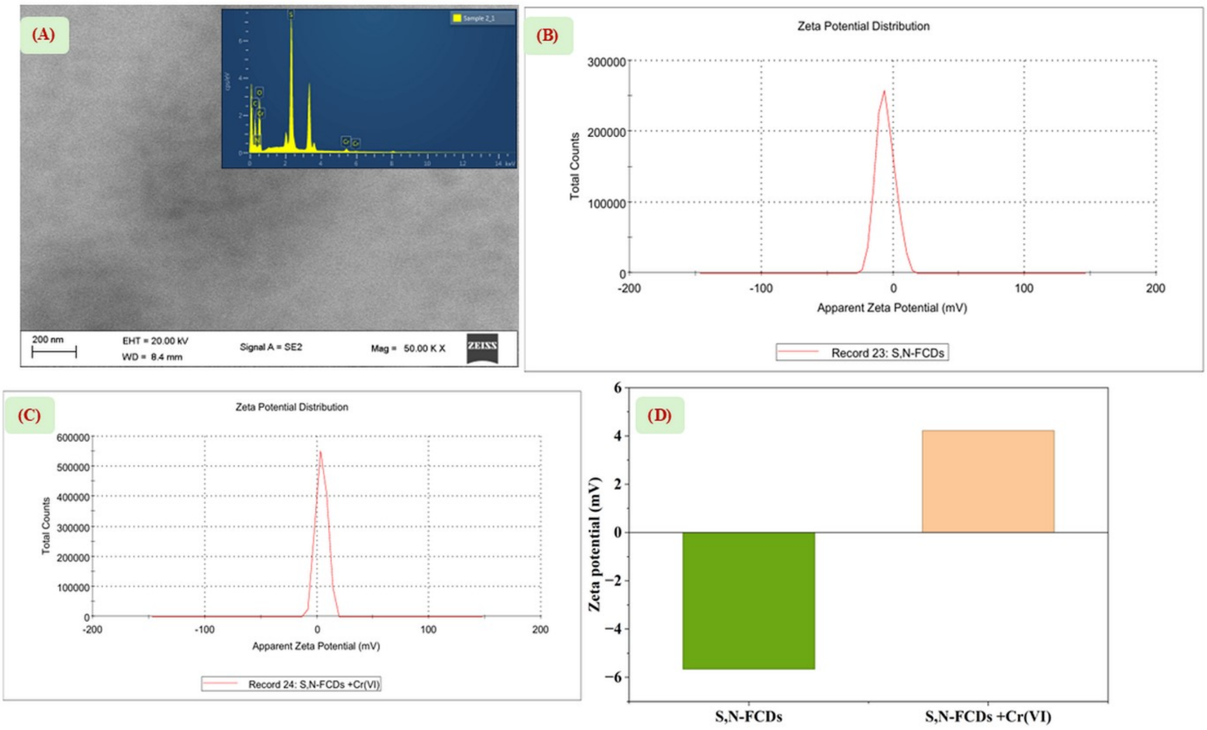

Figure S2. (A) *SEM micrographs of avocado seed-derived S, N-CDs mixed with 100 ppb of Cr(VI). (B & C) Zeta potential peak of S, N-CDs mixed with 20 μL of 10 mg/L of* $Cr_2O_7^{2-}$*, respectively (D) Bar graph of the Zeta potential of the S, N-CDs and mixture with* $Cr_2O_7^{2-}$.

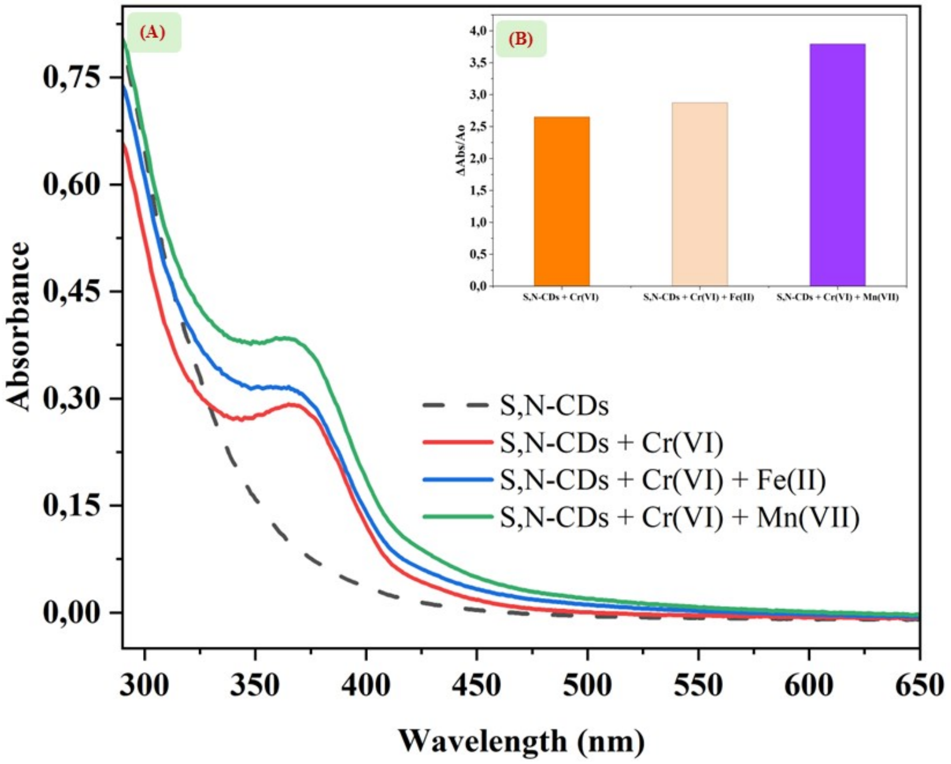

Figure S3. Overlaid *UV-visible absorption spectra of the S, N-CDs alone, 50 ppb Cr(VI) ions, and a mixture of 50 ppb Cr(VI +500 ppb (100-fold) Mn(VII) or Fe(II).*

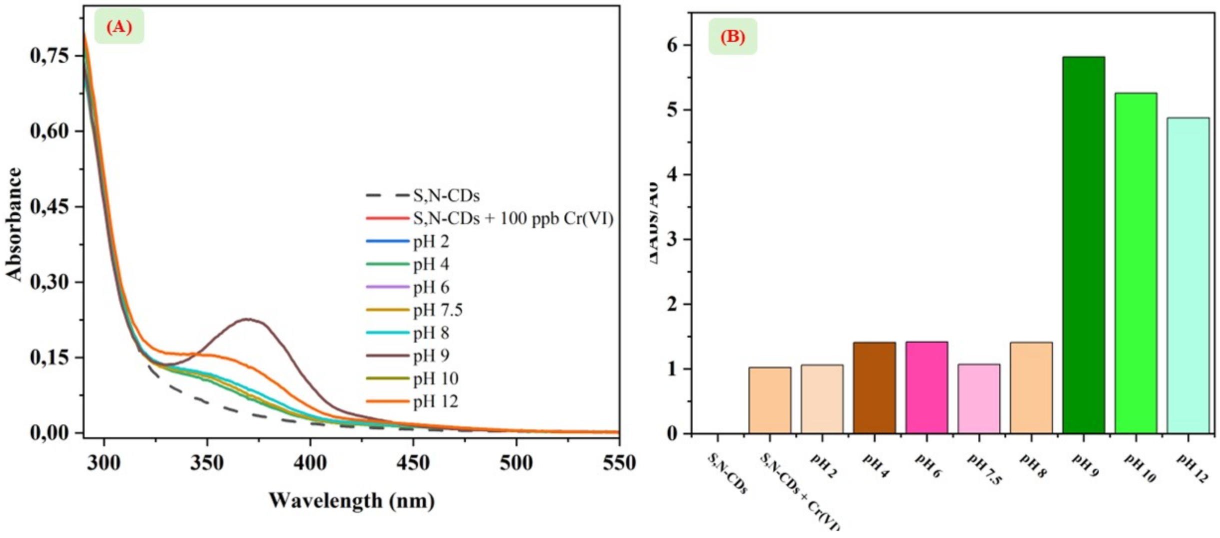

Figure S4. *(A) Overlaid UV-visible absorption spectra of 8.3 mg/L S, N-CDs (2.00 mL) when 20 μL of a 0.50 mg/L stock of was added at different pH values (2 - 12), 0.1 M NH_4OH adjusted with 0.1 M HCl or NaOH (B) Bar chart of the normalized absorbance responses at 375 nm.*

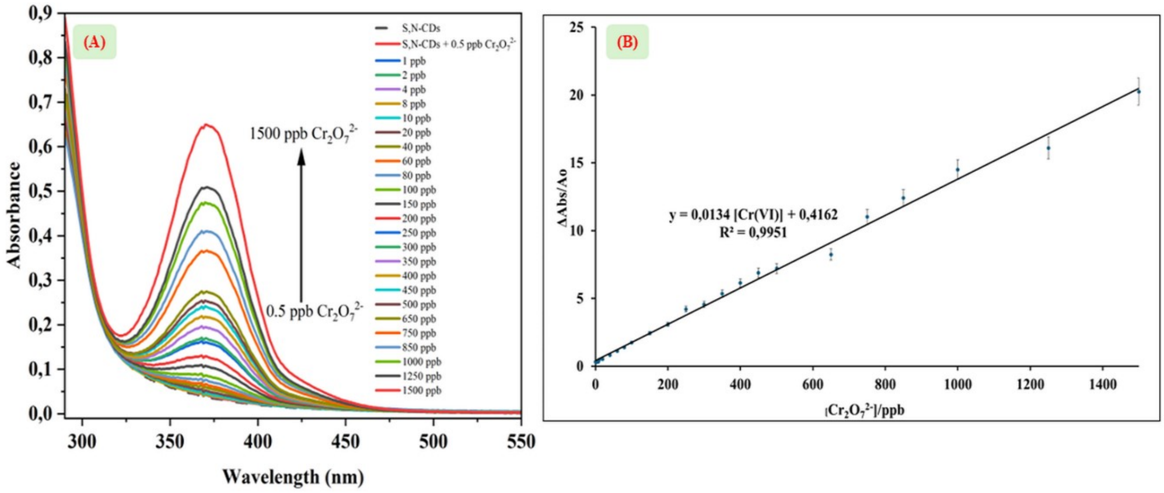

Figure S5. *(A) Overlaid UV-visible absorption spectra of the $Cr_2O_7^{2-}$ ions in S, N-CDs at pH 9 (ammonium buffer) when 20 μL of variable concentrations of $Cr_2O_7^{2-}$ ions were added to 1980 mL of the 8.3 mg/L S, N-CDs (2.00 mL) to attain final concentrations of 0.50 – 1500 ppb Cr(VI) using an old batch of S, N-CDs showing reproducibility. (B) Linear calibration plot of Cr(VI).*

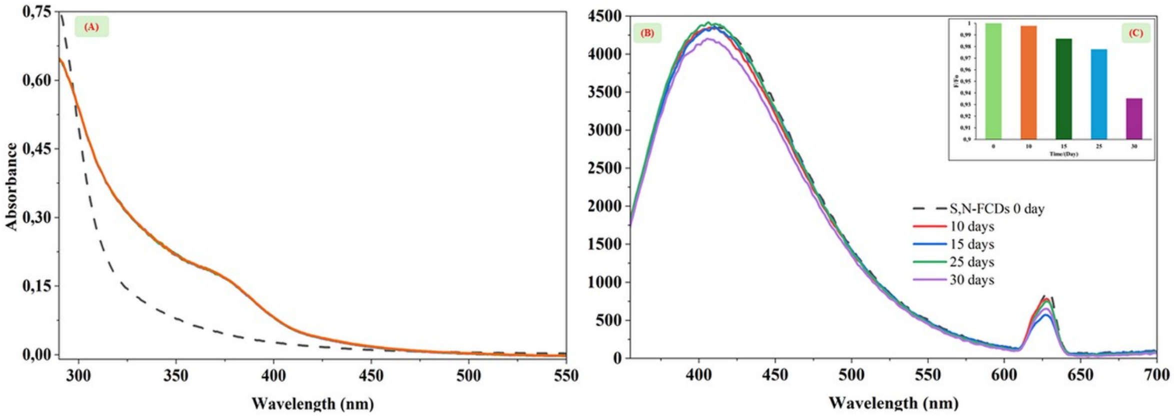

Figure S6. *Overlaid UV-visible absorption spectra of the CDs and after repeated scanning (N = 10) of S, N-FCDs (2.00 mL) mixed with 20 μL of $Cr_2O_7^{2-}$ [5.00 mg/L) to attain a final [$Cr_2O_7^{2-}$] of 50 ppb. The detection precision (reproducibility of the response) for detecting the Cr(VI) ion at* *a wavelength of 375 nm was calculated to be 2.7% RSD. (B) Inter-day measurements of the S, N-FCDs using FLUOR methods show only 6% decrease in intensity.*

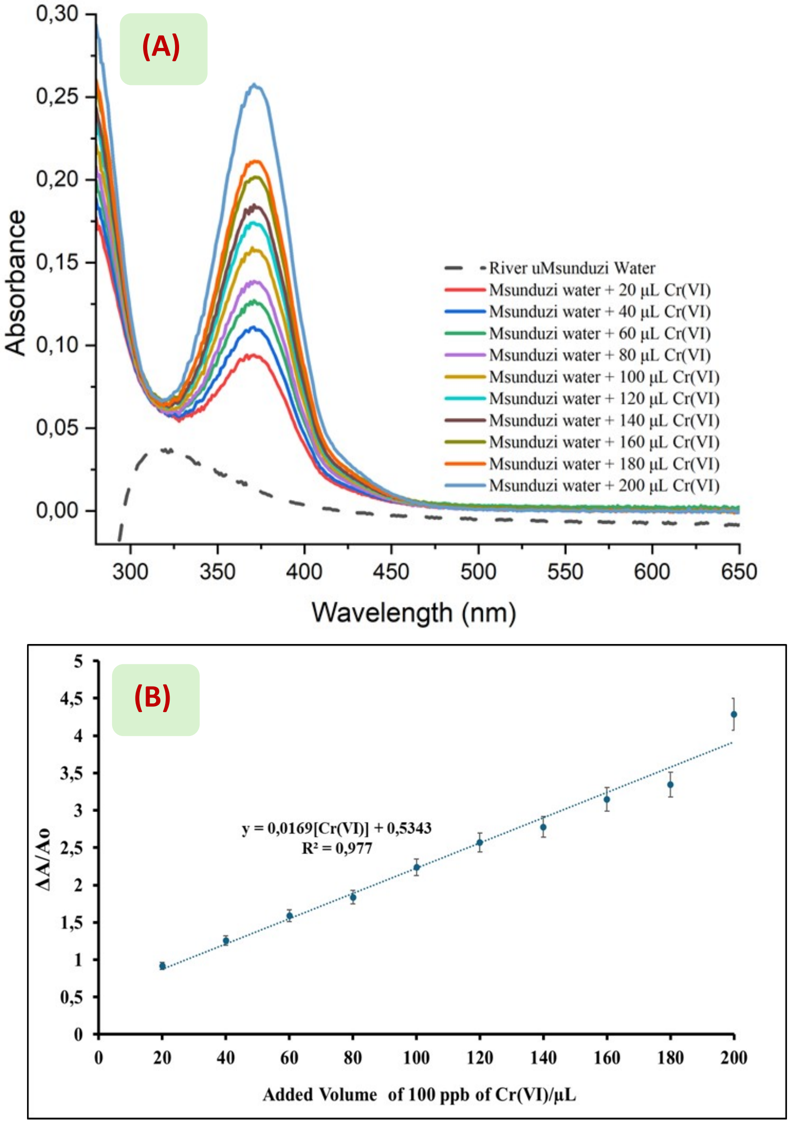

Figure S7. (*A) Overlaid UV-visible absorption of the water sample spiked with incremental volumes (20 µL) of the 200 ppb standard of Cr(VI) at 375 nm; the water was collected from the UMsunduzi River, Pietermaritzburg. (B) This is the standard addition calibration plot.*

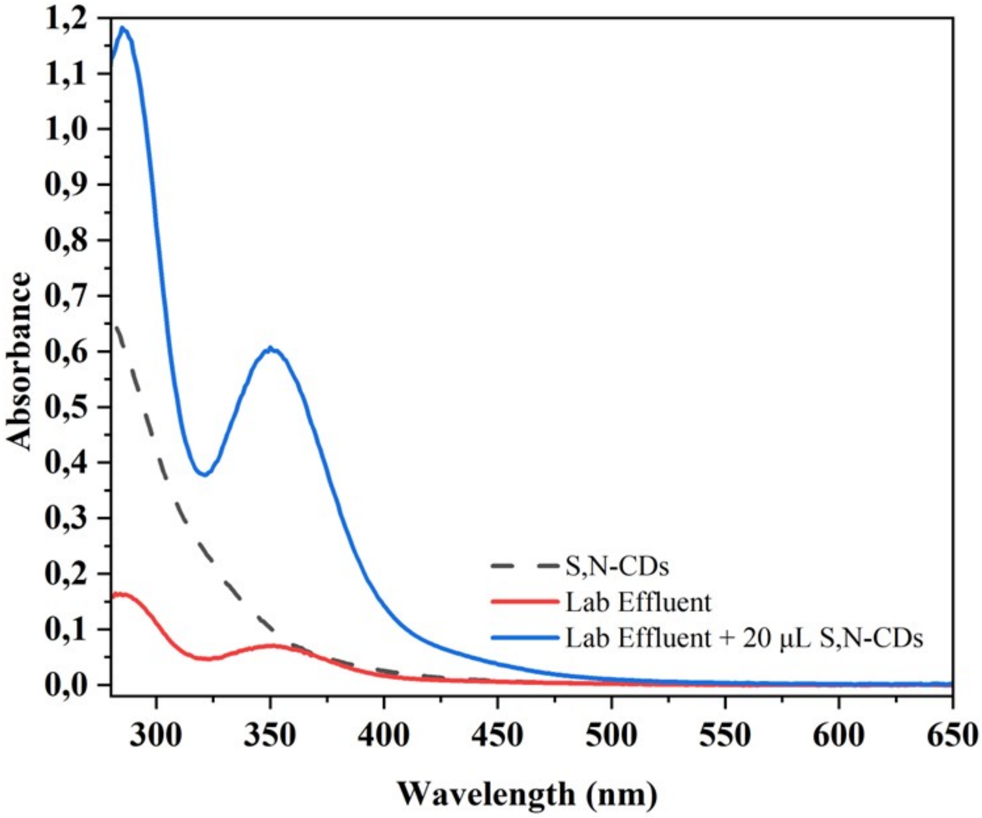

Figure S8. *Overlaid molecular absorption spectra of the S, N-CDs, laboratory wastewater sample, and its spike with Cr(VI) contaminated with Cr(VI showing the HGSIA effect.*

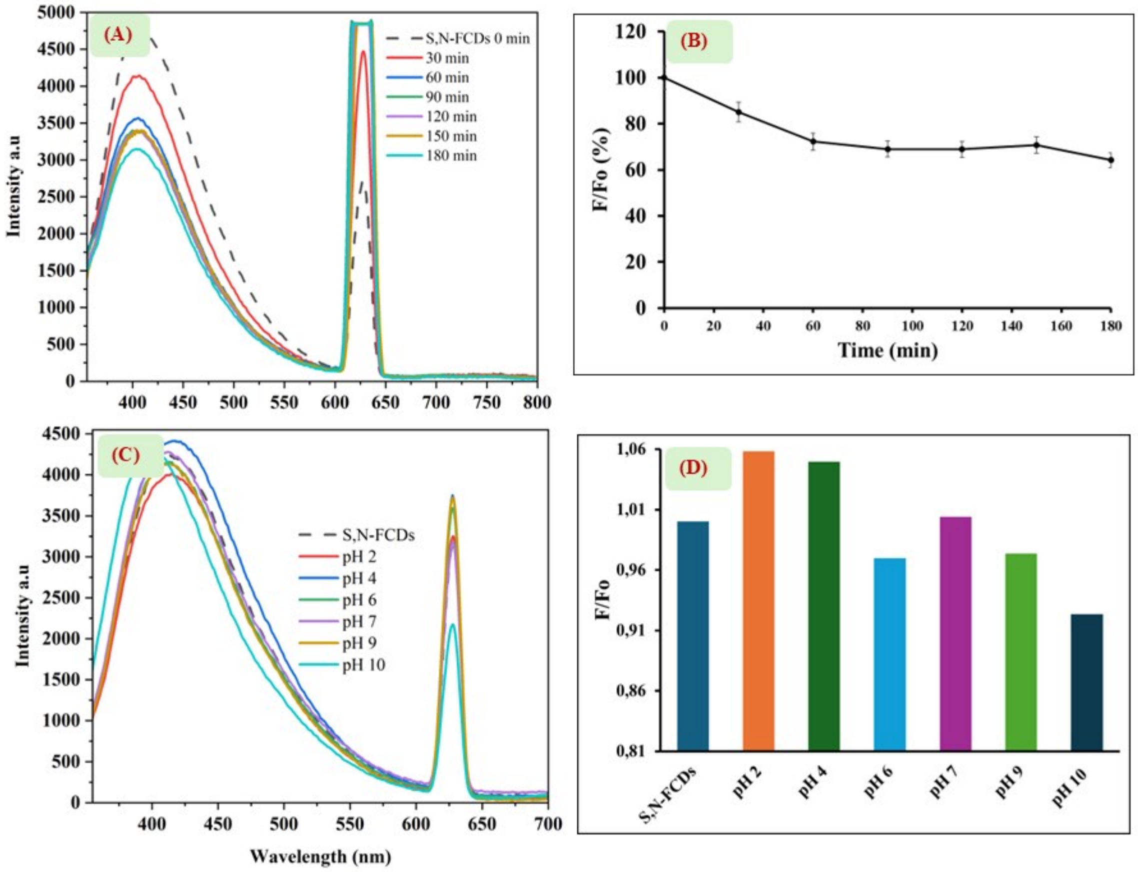

Figure S9. *(A) Overlaid fluorescence (FLUOR) spectra of S, N-FCDs collected at 30-minute intervals after irradiation with an 850 W UV-light lamp for 180 mins. (B) Photostability kinetic curve for the FLUOR of S, N-FCDs monitored at 420 nm. (C) Overlaid FLUOR spectra of pH-adjusted S, N-FCD solutions (ammonium buffer/0.1 M NH_4OH titrated with 0.1M HCl). (D) Normalized FLUOR (F/F_0) responses at 420 nm of the S, N-FCDs at different pH (2 - 10).*

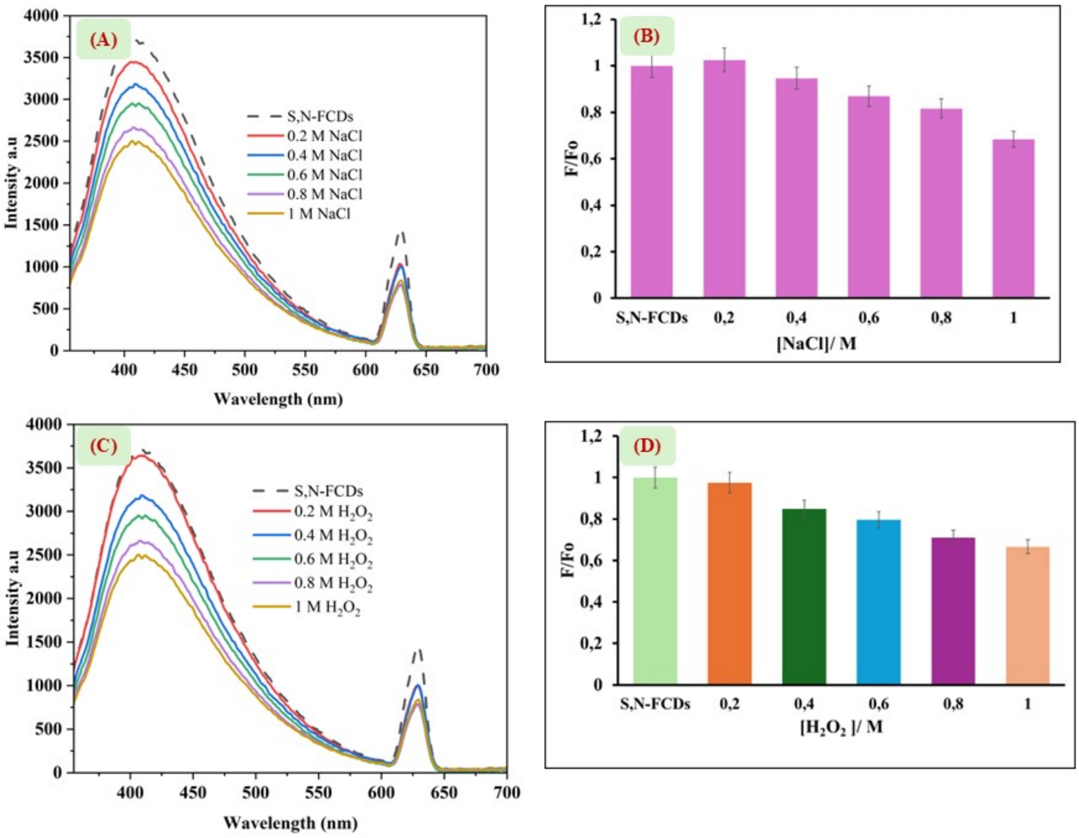

Figure S10. *(A) Overlaid fluorescence (FLUOR) spectra of S, N-FCDs in variable concentration (0.2 M - 1.0 M) NaCl. (B) Bar graphs of the normalized fluorescence(PL- F/F_0) of S, N-FCDs in (0.1 M - 1 M) NaCl at 420 nm (showing only a 31% quenching effect), decrease in intensity, showing high salt-resistivity. (C) Bar graphs of the normalized FLUOR responses (F/F_0) of the S, N-FCDs in (0.1 M - 1 M) in the presence of variable concentration (0.2 M – 1.0 M) of H_2O_2 monitored at 420 nm. (D) Overlaid FLUOR spectra of S, N-CDs in the presence of variable concentration (0.2 M - 1.0 M) of H_2O_2.*

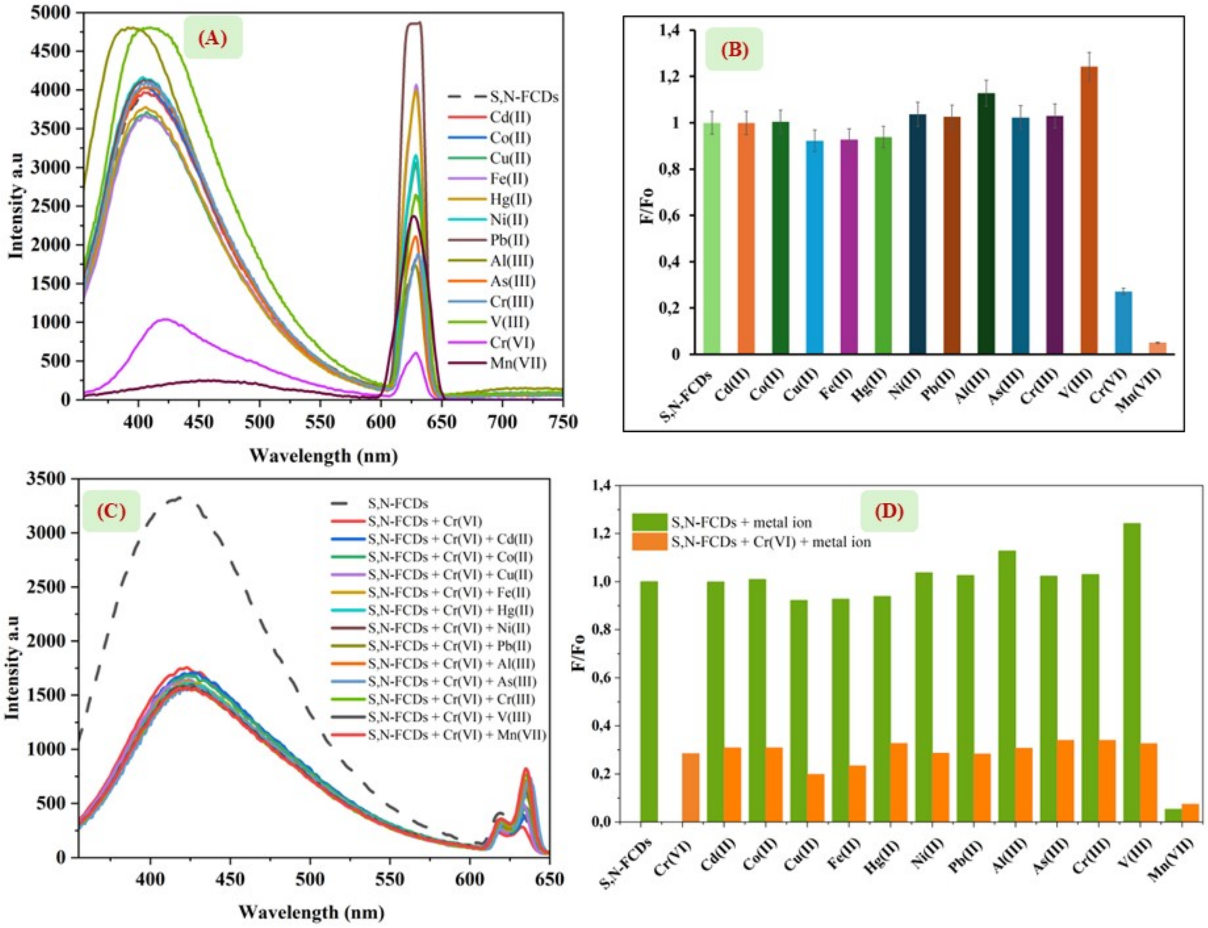

Figure S11. *(A) Overlaid fluorescence spectra of 8.3 mg/L avocado seed-derived S, N-CD solution (2.00 mL) before and after adding 20 µL 5.00 mg/L $Cr_2O_7^{2-}$ (Cr(VI)) or other metal ions) to 2.00 mL of the FCDs (to attain a final [$Cr_2O_7^{2-}$] or [metal ion] of 50 ppb). (B) Bar charts of the normalized changes in fluorescence (F/F_o) for the FLUOR-quenched solutions after separate additions of each metal ion {(Cr(VI), Cd(II), Cu(II), Fe(III), Ni(II), Cr(III), Al(III), Pb(II) and Mn(VII))}, where F_o is the fluorescence intensity of the 8.3 mg/L S, N-CDs solution and F is the FLUOR after adding each metal to attain a final [Cr(VI) + other metal ion] of 50 ppb. (C) Overlaid fluorescence spectra of 8.3 mg/L avocado seed-derived S, N-CD solution (2.00 mL) before and after adding 20 µL 5.00 mg/L $Cr_2O_7^{2-}$ (Cr(VI)) or other metal ions) to 2.00 mL of the FCDs (to attain a final [$Cr_2O_7^{2-}$] or [other metal ion] of 50 ppb. (D) Bar charts of the normalized changes in PL (F/F_o) for the fluorescence-quenched solutions after separate additions of each metal ion and Cr(VI), with an interferent ion at 420 nm.*

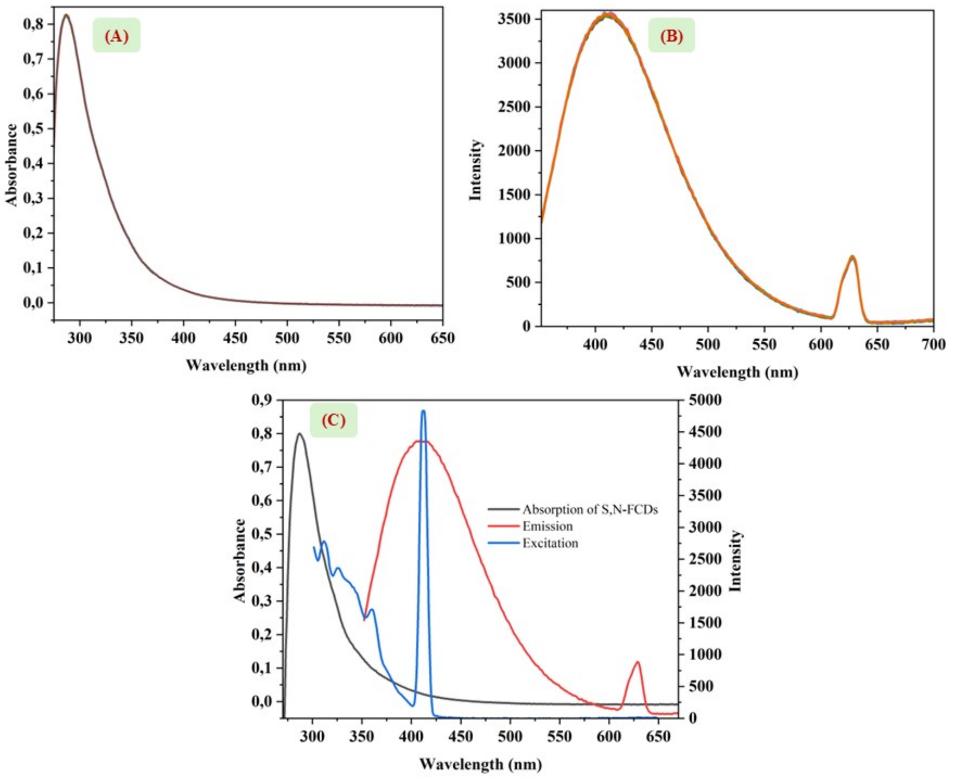

Figure S12. *Replicated (N = 20) absorption spectra of the (A) S, N-FCDs (reagent blank). (B) Replicated (N = 20) FLUOR spectra of S, N-FCDs (reagent blank). (C) Overlaid excitation, emission, and absorbance spectra of S, N-FCDs.*

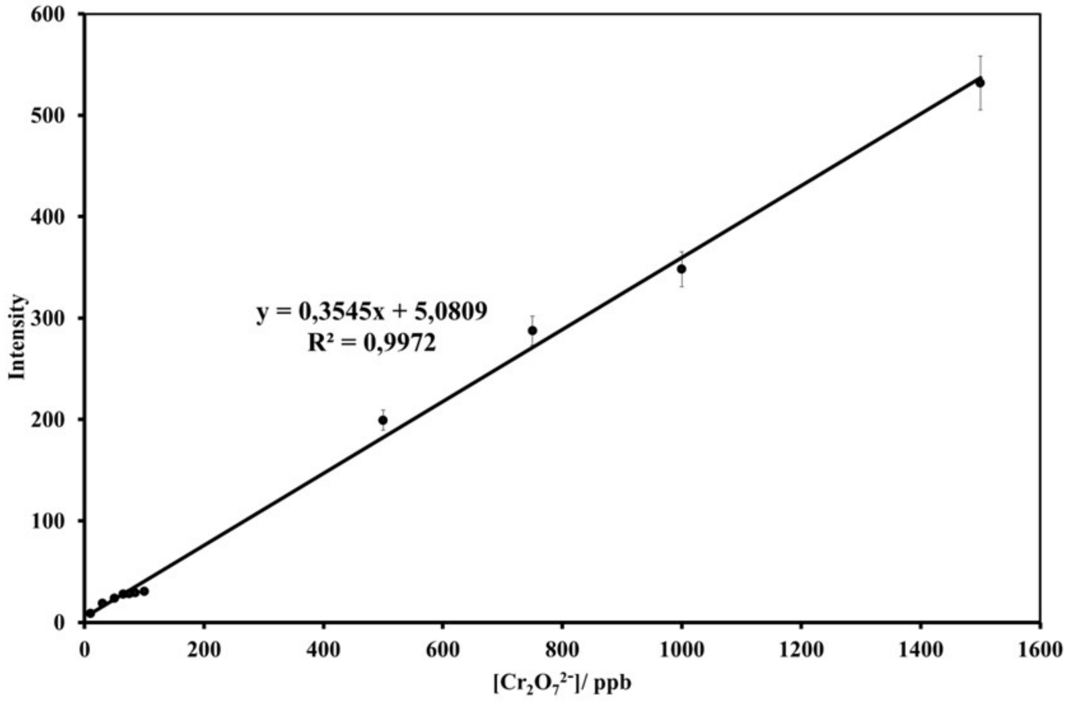

Figure S13. *ICP-OES Calibration curve of Cr(VI).*