

ARTICLE

1 **Polylactic acid-carbon nanofiber-based electro-conductive sensing material and paper-based**
2 **colorimetric sensor for detection of nitrates**

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30 (a)

S. No.	Exposure Concentration (mg/L)	Algal cell density		Total number of cells (72h-0h)	% Inhibition of Yield at 72 h
		0h	72h		
1	0	52542	1018750	966208	0
2	100	52542	1050000	997458	-3.23429

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S. No.	Exposure concentration (mg/L)	Mean of section-by-section growth rate at 72h	% Inhibition of growth rate 72 h
1	0	2.964349	0
2	100	2.994775	-1.02638

33 (b)

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35 **Supplementary Table 1.** Effects of CNF membrane on percent inhibition of growth rate (a) and
 36 Yield (b) of *Pseudokirchneriella subcapitata* after short-term exposure to limit dose (100 mg/L).

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S. No.	Test Organism	Exposure concentration	Duration	% Survival					Cumulative % Survival
				0 h	24 h	48 h	72 h	96 h	
1	Danio rerio (Zebrafish)	100 mg/L (Limit test)	96 h	100	100	100	100	100	100

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39 **Supplementary Table 2.** Effects of CNF membrane on the survival rate of adult zebrafish after
40 short-term exposure to limit dose (100 mg/L).

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S. No.	Test Organism	Exposure concentration	Duration	% Survival			Cumulative % Survival
				0 h	24 h	48 h	
1	<i>D. magna</i> (<24 h old daphnids)	100 mg/L (Limit test)	48 h	100 %	100 %	100 %	100 %

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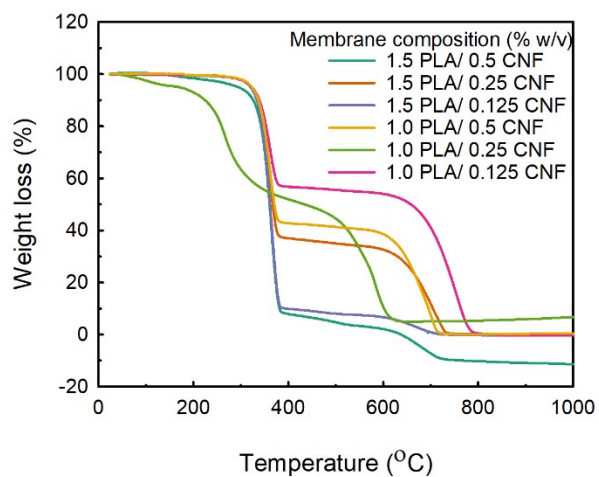
43 **Supplementary Table 3.** Effects of CNF membrane on the survival rate of Daphnids after Short
 44 term exposure to limit test concentrations (100 mg/L).

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S. No.	Existing technologies for the detection of nitrates	Linear range (ppm)	Detection limit (ppm)	Ref.
1	Flow-injection with luminol chemiluminescence detection	2×10^{-5} to 5×10^{-2}	2×10^{-5}	[1]
2	HPLC with UV absorption (HPLC/DAD)	5-35	4×10^{-3}	[2]
3	UV-Irradiated Photochemical Conversion to Peroxynitrite and Ion Chromatography-Luminol Chemiluminescence System	6×10^{-5} to 18×10^{-2}	1.8×10^{-3}	[3]
4	Electrothermal atomic absorption spectrometry	1×10^{-4} to 25×10^{-3}	1.3×10^{-5}	[4]
5	Two-electrode interdigitated sensors (IDEs)	4 to 14	4	[5]
6	UV spectroscopy	0.08 to 4.0	0.04	[6]
7	Ambient-pressure helium-plasma ionization-mass spectrometry (HePI-MS)	0.02 to 20	2×10^{-4}	[7]
8	Capillary electrophoresis technique	16.1 to 285	0.027	[8]
9	Present study (Electrochemical sensor)	78 to 5×10^3	0.046	
10	Present study (Paper-based colorimetric sensor)	1.56×10^2 to 10×10^3	1.56×10^2	

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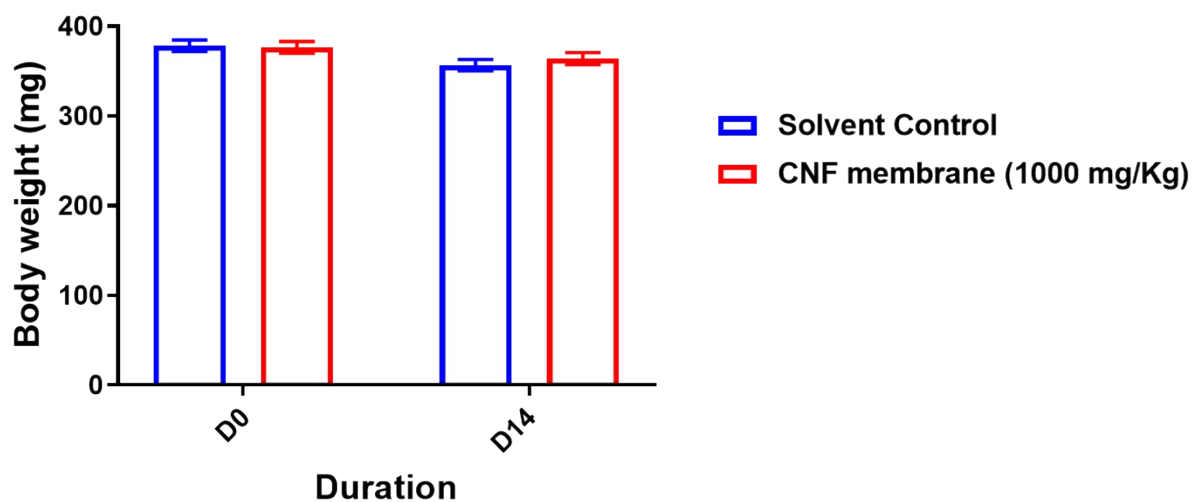
47 **Supplementary Table 4.** Comparative table of existing technologies and developed technologies for
 48 the detection of nitrates.



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50 **Supplementary Figure. 1:** TGA analysis of membranes composed of different concentrations of
51 PLA and CNF.

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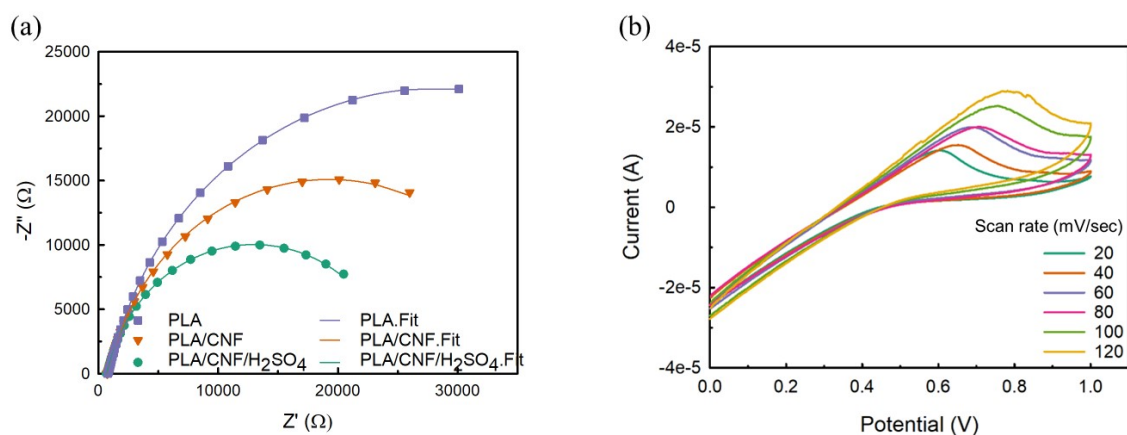


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55 **Supplementary Figure. 2.** No significant changes in body weight were observed ($p > 0.05$) in the
56 earthworm (*Eisenia fetida*) after short-term exposure to CNF membrane. Values expressed in
57 mean \pm SEM.

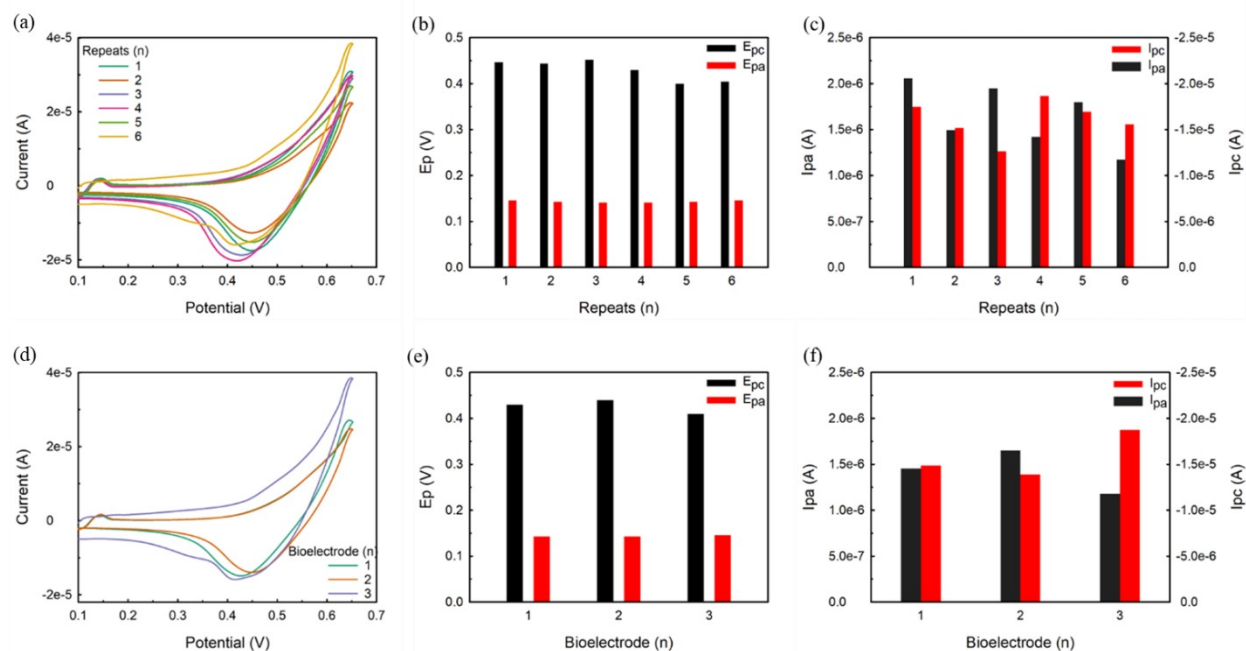
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60 **Supplementary Figure. 3:** Electrochemical behavior of membrane in the presence of one-electron
 61 redox system of $\text{Fe}(\text{CN})_6^{3-} / \text{Fe}(\text{CN})_6^{4-}$ (a) Nyquist plot shows reduced resistance to charge transfer
 62 on electrode surface after treatment with acid. PLA membrane had an R_{ct} of 36893Ω . The
 63 introduction of CNF to the membrane resulted in a decrease in the R_{ct} to 27135Ω . Further
 64 activation of PLA/CNF membrane with H_2SO_4 resulted in an R_{ct} of 19200Ω . A decrease in the
 65 R_{ct} value is indicative of the increased charge movement along the surface of the membrane. (b)
 66 Cyclic voltammogram of acid-activated PLA/CNF membrane at scan rate 20-120 mV s^{-1} showed
 67 an increase in I_p on increasing the scan rate.

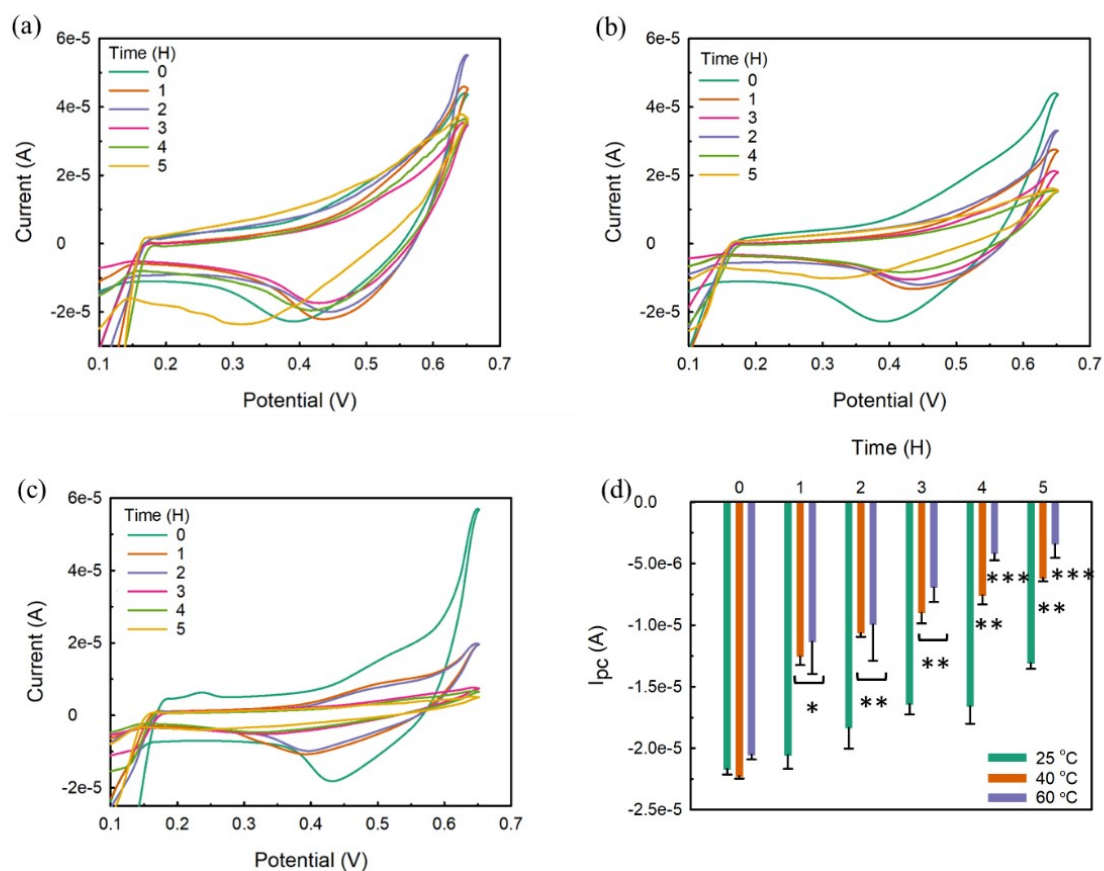
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70 **Supplementary Figure. 4:** (a) Cyclic voltammogram showing repeatability (n=6) of bio-based
 71 electrode tested with 10000 ppm of CAN in the presence of test solution. (b) Peak potential vs.
 72 repeat (n) showing non-significant variation in potential (E_{pa} : +0.15 V and E_{pc} : 0.42V). (c) Peak
 73 current vs repeat (n) showing non-significant variation in current (I_{pa} and I_{pc}). (d) Cyclic
 74 voltammogram showing reproducibility (n=3) of bio-based electrode tested with 10000 ppm of
 75 CAN in the presence of test solution. (e) Peak potential vs. bio-based electrode (n) showing non-
 76 significant variation in potential (E_{pa} : +0.15 V and E_{pc} : 0.42V). (f) Peak current vs bio-based
 77 electrode (n) showing non-significant variation in current (I_{pa} and I_{pc}).

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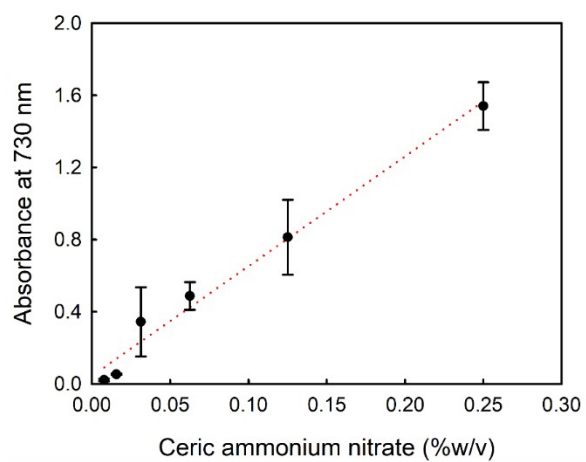


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81 **Supplementary Figure. 5:** Cyclic voltammogram of bio-based electrode in the presence of test
 82 solution after storage at (a) 25, (b) 40 and (c) 60 °C. (d) Stability of the bio-based electrode tested
 83 with 10000 ppm of CAN where *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

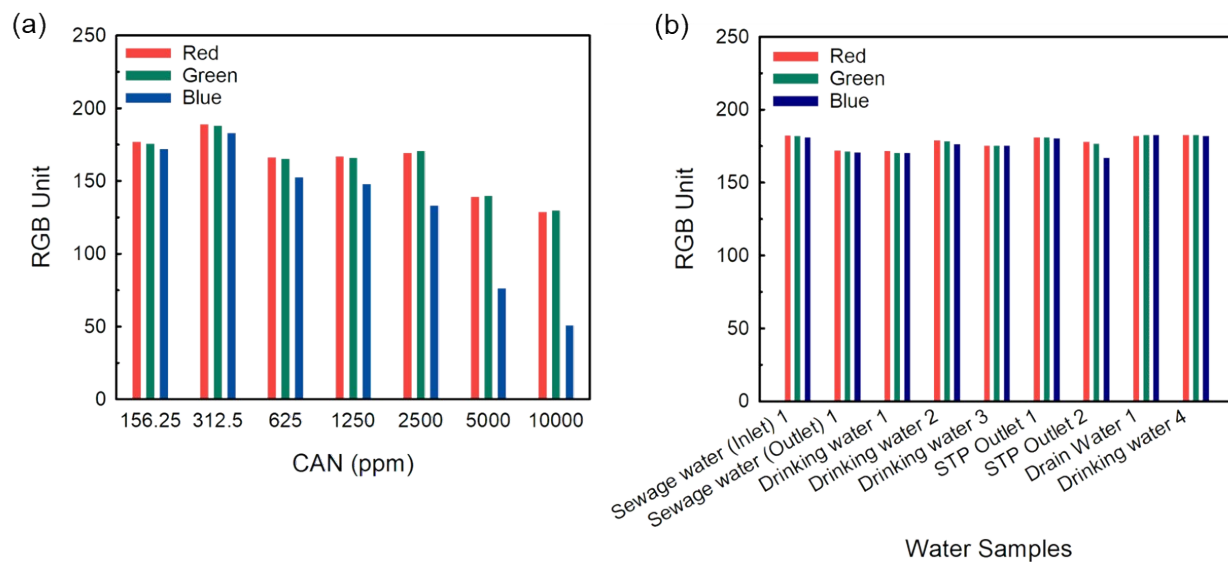
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86 **Supplementary Figure. 6:** Dose-response of test solution and Ceric ammonium nitrate observed
87 at 730 nm.

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90 **Supplementary Figure. 7:** RGB analysis of chromogenic test strips tested with (a) ceric
91 ammonium nitrate standards and (b) water samples.

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94 **References**

- 95 [1] M. Yaqoob, B. Folgado Biot, A. Nabi, P.J. Worsfold, Determination of nitrate and nitrite in freshwaters
96 using flow-injection with luminol chemiluminescence detection, *Luminescence*, 27 (2012) 419-425.
- 97 [2] M. Tatarczak-Michalewska, J. Flieger, J. Kawka, W. Plazinski, W. Flieger, E. Blicharska, D. Majerek,
98 HPLC-DAD Determination of Nitrite and Nitrate in Human Saliva Utilizing a Phosphatidylcholine
99 Column, *Molecules*, 24 (2019).
- 100 [3] H. Kodamatani, S. Kubo, A. Takeuchi, R. Kanzaki, T. Tomiyasu, Sensitive Detection of Nitrite and
101 Nitrate in Seawater by 222 nm UV-Irradiated Photochemical Conversion to Peroxynitrite and Ion
102 Chromatography-Luminol Chemiluminescence System, *Environmental Science & Technology*, 57
103 (2023) 5924-5933.
- 104 [4] R. Roohparvar, T. Shamspur, A. Mostafavi, H. Bagheri, Indirect ultra-trace determination of nitrate and
105 nitrite in food samples by in-syringe liquid microextraction and electrothermal atomic absorption
106 spectrometry, *Microchemical Journal*, 142 (2018) 135-139.
- 107 [5] M.E.E. Alahi, N. Afsarimanesh, S.C. Mukhopadhyay, L. Burkitt, Development of the selectivity of
108 nitrate sensors based on ion imprinted polymerization technique, 2017 Eleventh International
109 Conference on Sensing Technology (ICST), 2017, pp. 1-6.
- 110 [6] A.C. Edwards, P.S. Hooda, Y. Cook, Determination of Nitrate in Water Containing Dissolved Organic
111 Carbon by Ultraviolet Spectroscopy, *International Journal of Environmental Analytical Chemistry*, 80
112 (2001) 49-59.
- 113 [7] J. Pavlov, A.B. Attygalle, Direct Detection of Inorganic Nitrate Salts by Ambient Pressure Helium-
114 Plasma Ionization Mass Spectrometry, *Analytical Chemistry*, 85 (2013) 278-282.
- 115 [8] N. Kamilova, Z. Kalaycıoğlu, A. Gölcü, Sample Stacking–Capillary Electrophoretic Analysis of Nitrate
116 and Nitrite in Organic- and Conventional-Originated Baby Food Formulas from Turkey, *ACS Omega*,
117 8 (2023) 5097-5102.

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