

1 **SUPPLEMENTARY MATERIALS**2 **Supplementary Table 1. Summary of the elements from the two sampling sites based on ICP-OES analysis**
3 **(n=3)**

Elements	Units	Al	K	Pb	Mn	Cd	Fe
N. C.	mmol/L	0.004 ± 0.004	0.276 ± 0.009	n.a.	0.001 ± 0.0003	n.a.	0.011 ± 0.001
	mg/L	0.106 ± 0.110	10.790 ± 0.354	n.a.	0.051 ± 1.564	n.a.	0.597 ± 0.054
C. C.	mmol/L	0.190 ± 0.024	0.824 ± 0.074	n.a.	0.028 ± 0.003	n.a.	1.163 ± 0.139
	mg/L	4.378 ± 0.655	32.211 ± 2.922	n.a.	1.564 ± 0.167	n.a.	64.937 ± 7.761
		Mg	Na	Co	Zn	Cu	Cr
N. C.	mmol/L	0.161 ± 0.018	0.452 ± 0.028	n.a.	n.a.	0.003 ± 0.001	n.a.
	mg/L	3.913 ± 0.433	10.38 ± 0.655	n.a.	n.a.	0.162 ± 0.089	n.a.
C. C.	mmol/L	2.540 ± 0.254	2.560 ± 0.261	0.006 ± 0.001	0.005 ± 0.0004	0.016 ± 0.001	n.a.
	mg/L	61.725 ± 6.128	58.848 ± 6.005	0.380 ± 0.064	0.301 ± 0.029	0.998 ± 0.092	n.a.
		Ca	Ni	S	P	As	Cl
N. C.	mmol/L	0.258 ± 0.038	0.005 ± 0.001	0.848 ± 0.022	0.027 ± 0.0001	n.a.	0.001 ± 0.000
	mg/L	10.321 ± 1.518	0.268 ± 0.045	n.c.	n.c.	n.a.	n.c.
C. C.	mmol/L	6.393 ± 0.878	0.184 ± 0.019	13.856 ± 1.53	0.026 ± 0.001	n.a.	0.002 ± 0.000
	mg/L	256.235 ± 35.196	10.805 ± 1.118	n.c.	n.c.	n.a.	n.c.

4 N.C. – Nolin Creek; C.C. - Copper Cliff.

5 n.a. entry represents data below detection limit or below 1 µmol/L; n.c. entry was not calculated.

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7 **Supplementary Table 2. Nickel fractionation data (corresponding to Test 2)**

Fraction	pCDF-Duet (Empty Vector)				Ni_v.1			
	+IPTG		-IPTG		+IPTG		-IPTG	
	% Ni	mg _{Ni}	% Ni	mg _{Ni}	% Ni	mg _{Ni}	% Ni	mg _{Ni}
Biomass ^a	13.5	0.015	29.9	0.032	23.2	0.025	28.0	0.030
	± 1.5	± 0.001	± 2.2	± 0.002	± 1.6	± 0.002	± 3.2	± 0.004
Wash ^b	35.6	0.040	32.0	0.035	23.0	0.025	31.8	0.034
	± 0.4	± 0.001	± 1.9	± 0.002	± 0.8	± 0.002	± 2.9	± 0.003
Residual	50.9	0.057	38.1	0.041	53.7	0.058	40.2	0.043
	± 1.5	± 0.002	± 1.7	± 0.002	± 2.2	± 0.001	± 9.3	± 0.001

8 Percentage nickel found in each fraction was calculated by dividing the mg_{Ni} in each fraction by the sum of the mg_{Ni}
9 measured across all fractions for each strain (empty vs. Ni_v.1) and condition (IPTG).10 ^a – the amount of nickel bioaccumulated into the cell; ^b – the amount of nickel biosorbed on the surface of the cell

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13 Supplementary Table 3. **Primers**

ID	Primer Name	Sequence (5' to 3')	T_m (°C)	Purpose
0	Forward NikABCDE	gtacgaccatggatgctctccacactcggccgcactc	65	Cloning
1	Reverse NikABCDE	gtacgaaagctttaaacctttctgtggtgcgacggcgca	65	
2	Forward MTA	gtacgacatatgagcggctcgggtgcggcag	65	Cloning
3	Reverse MTA	gtacgattaattaacttacagttgcagggatcacaggtgcaattatcaccgc	65	
4	Forward Site 1 Sequencing	ggatctcgacgctctccct	57	Sequencing
5	Reverse Site 1 Sequencing	gattatgcgggcgtgtacaa	55	
6	Forward Site 2 Sequencing	ttgtacacggccgcataatc	55	Sequencing
7	Reverse Site 2 Sequencing	gctagtattgtctcagcgg	53	

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15 Supplementary Table 4. **Sequences**

Sequence Name	Link to Sequences^b
pCDF-Duet Vector	https://benchling.com/s/seq-PblPbPGI12SVQzCKoDEF?m=slm-SB4QqukyRhd6Wndg6xTJ
MTA Only	https://benchling.com/s/seq-UbCXMDVrU3IJEIYdKr76?m=slm-0qaIb0Xpj0AbycIyurIc
NiKABCDE Only ^a	https://benchling.com/s/seq-mWaP9Yy6MfSXuzQu8F7k?m=slm-0oHRIjWjtp56UXqM0cjd
Full Ni_v.1 Construct ^a	https://benchling.com/s/seq-JyRqP9nA1wXe417p1Acq?m=slm-SIvaxbfhQWLuo9YHK09f

16 ^a An additional base pair leading to a frameshift mutation for NikABCDE and the full Ni_v.1 construct was
 17 corrected (*i.e.*, removed) by Ranomics through site directed mutagenesis prior to all testing.

18 ^b If the URL links do not work, please contact the corresponding author.



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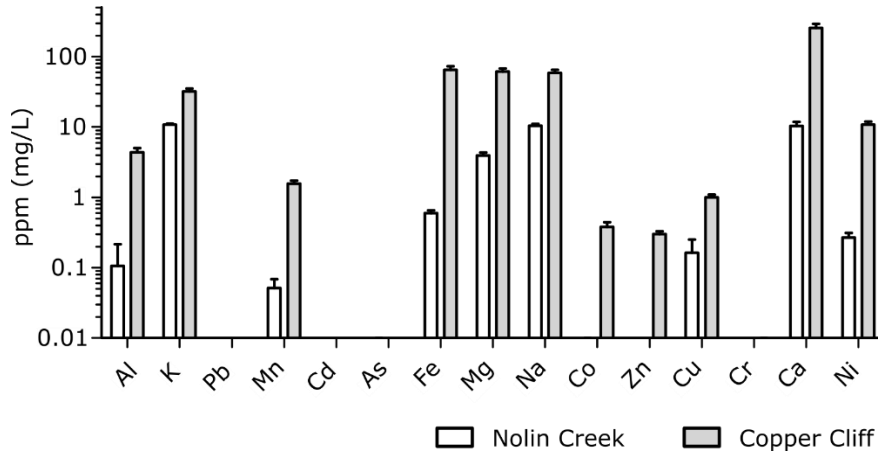
20 **Supplementary Figure 1. Aerial view of aqueous stream sampling sites.** Blue star – Copper Cliff (C.C.). Pink star –
21 Nolin Creek (N.C.). (A) View of Vale Copper Cliff site and the City of Greater Sudbury. (B) View of the C.C. site.
22 (C) View of the N.C. site. Aerial images taken from Google Earth (accessed on July 2022).

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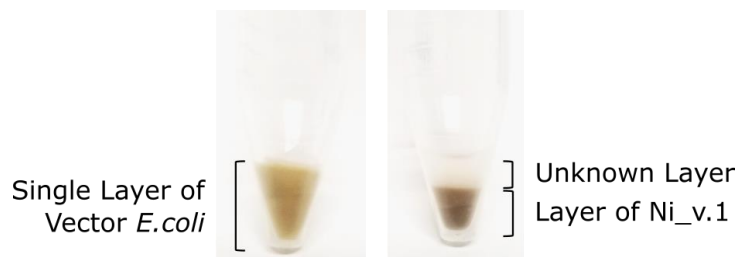


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28 Supplementary Figure 2. **Metal content comparison between sampling sites.** Same data displayed in Figure 3 of
 29 main text, but with metal concentrations for each metal plotted side-by-side for easier comparisons.

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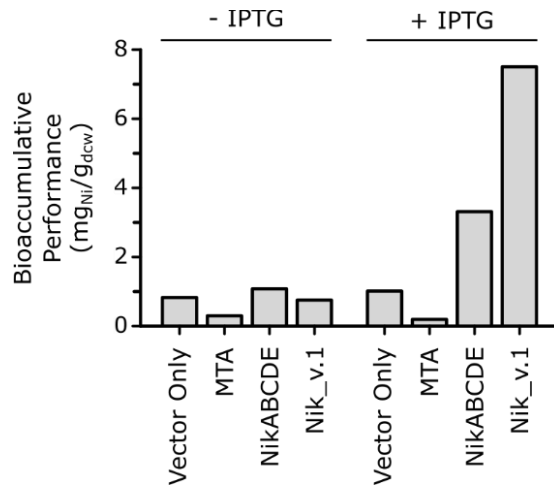


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33 Supplementary Figure 3. **Comparison of pellet appearance before acid digestion at the end of T5.** Vector Only
 34 control (left) and Ni_v.1 (right) pelleted via centrifugation in 15 mL conical vials.

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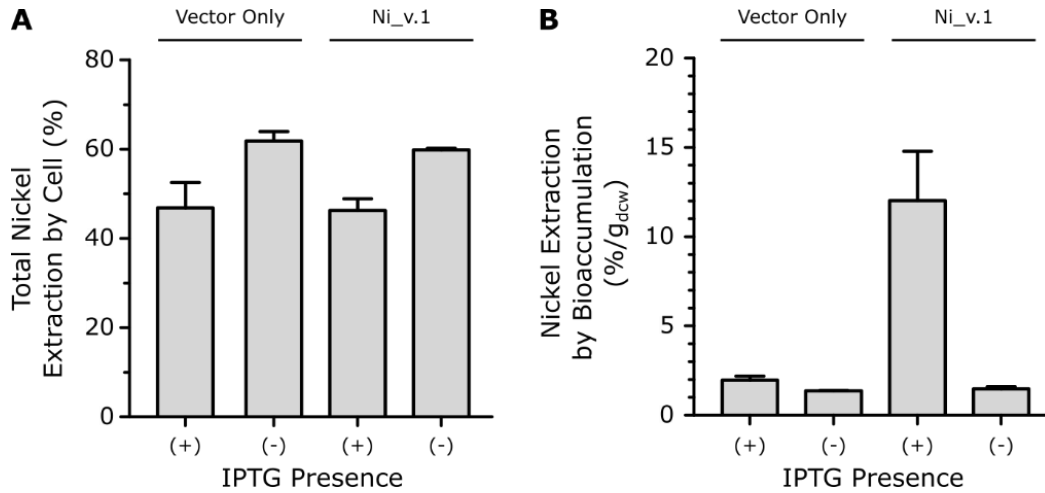
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38 Supplementary Figure 4. **Ni_v.1 bioaccumulation performance compared to controls.** Single replicate flask
 39 experiments for four strains were tested in the absence and presence of 0.4 mM IPTG for induction: Vector Only

40 (empty pCDF-Duet vector where both storage and transport systems were absent), MTA (only the storage system was
 41 present), NikABCDE (only the transport system was present), and Nik_v.1 (where both the transport and storage
 42 systems were present).

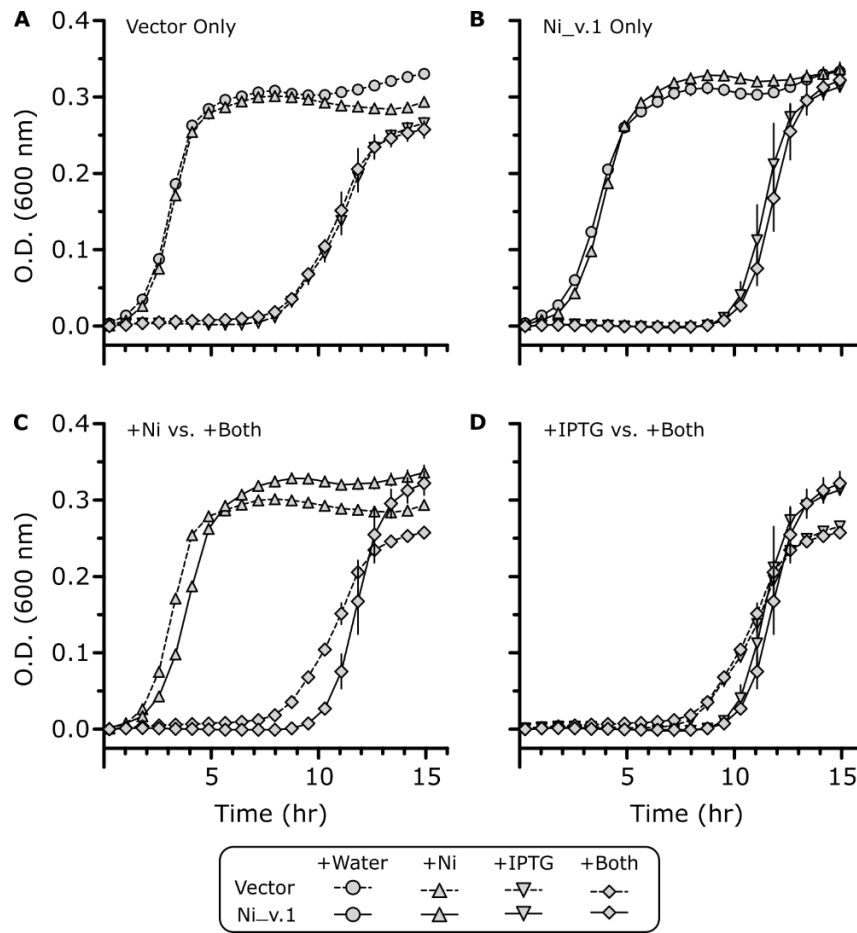


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44 Supplementary Figure 5. **Nickel extraction performance.** Vector Only, referring to the strain carrying the empty
 45 pCDF-Duet vector, and Ni_v.1 referring to the strain carrying both NikABCDE and MTA. (A) Percent nickel
 46 extraction based on the total nickel (mg_{Ni}) measured across all three fractions (residual, wash, and biomass). (B)

47 Percent nickel extractions normalized to the amount of cells (g_{DCW}) obtained in the biomass fraction. Experiments
 48 were performed in triplicate ($n=3$).

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51 Supplementary Figure 6. **Assessment of Ni_v.1 growth inhibition by nickel and IPTG.** Growth curves are plotted
 52 to compare the effects of 10 ppm NiCl₂, 0.4 mM IPTG, or both for (A) Vector Only controls, and (B) Ni_v.1 samples.
 53 The synergistic effects of nickel (C) and IPTG (D) are compared ($n=3$). Every other three datapoints were plotted to
 54 avoid cluttering.

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