

Section S1: Preparation of HM and AB Initial Stocks

Initial stocks of metal solutions were made in distilled water to reach the concentrations of 1 M for Cd and Hg ions, and 5 M for Zn ions, using CdCl₂ (MW=183.31 g mol⁻¹), ZnSO₄·7H₂O (MW=287.55 g mol⁻¹) and HgCl₂ (MW=271.52 g mol⁻¹). The pH of metal solutions was adjusted to neutral with 0.1 M NaOH and solutions were autoclaved (121 °C, 15 min, 1 bar). These were used as source solutions for HM additives to media to culture soil bacteria. The high water-solubility of these HM's salts made them suitable options for bacterial culture-based experiments [1-5].

Five different Ab of various Ab groups, including Tetracycline (Tc), Chloramphenicol (Cm), Carbenicillin (Cb), Erythromycin (Ery) and Ampicillin (Amp) were used to prepare the Ab stocks. These Ab are members of the tetracyclines, chloramphenicol, antipseudomonal penicillins, macrolides and penicillins groups respectively. Initial stock concentrations of 0.2 g mL⁻¹ of these Ab were prepared in dimethyl sulfoxide (DMSO). The Ab stocks were sterilised by passing them through sterile 0.45 µm syringe filters and stored at -20 °C [6, 7].

The following equation was used to calculate the volume of 1 or 5 molar initial metal compound solution to be added per L kg⁻¹ of media/soil:

$$i = \frac{X \times 1000000}{1000 \left(\frac{M}{C} \right)}$$

where i is the volume of metal compound solution (mL) to be added to one litre of media or one kg of soil, X is the preferred final concentration of metal in media/soil by mM, M is the molarity of metal's initial stock, C is the percentage of metal atoms in its 1 M compound solution.

Section S2: CFU Calculation

The mean values from each set of three replicates were calculated and total CFU obtained per g of soil dry mass using the following equation:

$$CFU_{DM} = \frac{N \times D \times 100}{TW_w - SW_w}$$

where CFUDM is CFU per soil dry mass, N is number of colonies per plate, D is inverse dilution factor, TW_w is total weight of soil, which is for example 1 g, and SW_w is the weight of water in the soil. The total CFU (CFUT) in 1 g of soil was determined using the following equation:

$$CFU_T = \frac{CFU_{DM}}{TW_w}$$

Section S3: Bacterial Cell Density Adjustment

The density of bacterial inoculum was measured and set spectrophotometrically —OD=0.1 at 600 nm wavelength— at 1×10⁸ cells by adding adequate amount of fresh R2A broth. Spectrophotometer OD measurement validation was performed using a McFarland 0.5 turbidity standard. The turbidity standard solution was made by mixing 1 mL of 48mM barium chloride dihydrate (BaSO₄·2H₂O) solution with 199 mL of 1% sulphuric acid (H₂SO₄) and shaking vigorously. Its optical density (OD) was measured using both spectrophotometer and plate reader at 600 nm and showed 0.1 absorbance, proving both the prepared standard turbidity solution and spectrophotometer OD measurement accuracy [8].

To reach the desired number of bacterial cells in liquid culture of 5×10⁵ mL⁻¹, 1:200 dilution of stationary phase of bacterial cultures were prepared —100 µL of liquid culture with OD=0.1 in 19.9 mL of fresh liquid media [9, 10]. Since the OD absorption can be different for various bacterial isolates with

different cell size, shape and colour, the precise number of colonies mL⁻¹ at a given OD for each bacterial isolate was determined; therefore, a 10 µL aliquot of each 1:200-diluted liquid culture was added to 990 µL fresh R2A broth and a 100 µL aliquot was spread on the surface of basal R2A agar. The number of colonies was counted (between 10-300 colonies) and CFU was determined for the 100 µL-volume positive control at the time zero point using the following equation:

$$CFU = \frac{C \times 10}{10^{-D}}$$

Where *CFU* is the total number of individual colonies grown from 100 µL of liquid culture; *C* is number of colonies per plates; 10^{-D} represents the number of 10-fold dilution of liquid culture which is *D*=2 here; 10 (µL) represents the liquid culture aliquot volume diluted by 1:100. This scenario helped to monitor the precise number of individual cells/CFU of each bacterial isolate that were added to the microtiter wells [8].

Section S4: Preparation of HM and Ab Stock Solutions

To measure the MIC (the lowest concentration of antimicrobial agents inhibiting visible planktonic cell growth), the desired range of HM and Ab concentrations were selected based on literature reports and standard MICs for bacterial isolates reported by EUCAST respectively [11]. Stock solutions 1000X stronger than required final concentrations of HM and Ab were prepared; these concentrations included 0.1, 0.5, 1, 5, 10, 20, 100, 200 and 500 µg mL⁻¹ (dissolved in DMSO) for Ab . Final HM ion concentrations of 0.0001, 0.001, 0.01, 0.1, 1, 2, 5 and 10 mM of Cd using CdCl₂, 0.001, 0.01, 0.1, 1, 2, 5, 10 and 20 mM of Zn using ZnSO₄·7H₂O, and 0.00001, 0.0001, 0.001, 0.01, 0.1, 1, 2 and 5 mM of Hg using HgCl₂, were prepared in sterile 1.5 mL polypropylene micro-centrifuge tubes [12].

Section S5: Growth Monitoring

Agar plate culturing of the liquid growth in the microtiter plates was performed on R2A plates, in order to monitor the plate reader OD accuracy, and also the growth of the bacterial population in the presence of HM and Ab after 72 h incubation [193]. Aliquots (100 µL) from each triplicate well were mixed and an aliquot of 100 µL of the cell mixture was cultured on basal R2A agar plates, after suitable dilution based on their OD. The equation described in Section S2 was used to calculate the number of bacterial CFU in each culture. The agar plate culturing helped to monitor and compare between the number of viable versus senescent bacterial cells in the microtiter plates liquid culture [8]. The positive and negative controls mentioned in Section 2.3, were used here too.

Section S6: Measuring HM Bioavailability in Microtiter Plate Wells

Due to the supposition that absorption to the surface of microtitre plates wells by some HM ions might have occurred, HM bioavailability in polystyrene microtiter plate wells was measured after the incubation time using Atomic Absorption Spectrophotometry (AAS). After mixing triplicate wells including those for the positive and negative controls, and inoculated wells with each concentration of HM , bacterial cells were precipitated by spinning at 3000 rpm for 5 minutes. The supernatants after serial dilution detectable by the AAS, were applied to the machine.

Table S1. Methods used to determine the physicochemical features of Waikato Region soil samples.

Test	Method Description
Soil Preparation	Air dried at 35-40 °C overnight (residual moisture typically 4%).
pH	1:2 (v/v) soil:water slurry followed by potentiometric determination of pH.
Olsen Phosphorous	Olsen extraction followed by Molybdenum Blue colorimetry.
Metals extensive suite, trace level (33 metals)	Dried sample, <2 mm fraction. Nitric/Hydrochloric acid digestion, ICP-MS, trace level.
Total Recoverable digestion	Nitric/hydrochloric acid digestion. US EPA 200.2.
Total Fluoride in solids alkaline fusion	Alkaline fusion of sample. Methods of Soil Analysis 2nd Edition, Pt2, 26-4.3.3.
Total Fluoride in Solids	Ion selective electrode. Methods of Soil Analysis 2nd Edition, Pt2, 26-4.3.3.
Total Organic Carbon	Acid pretreatment to remove carbonates present followed by Catalytic Combustion (900 °C, O ₂), separation, Thermal Conductivity Detector [Elementar Analyser].

Table S2. PCR conditions to amplify desired DNA fragments for TRFLP analysis

Step	Temperature °C	Time	Number of cycles
Initial denaturation	95	3 min	1
Denaturation	95	30 sec	
Annealing	55	30 sec	30
Extension	72	1 min	
Final extension	72	20 min	1

Table S3. PCR conditions to amplify Cd resistant encoding genes

Gene	Step	Temperature °C	Time	Number of cycles
<i>czcA</i>	Initial denaturation	94	5 min	1
	Denaturation	94	30 sec	
	Annealing	58	1 min	35
	Extension	72	1 min	
	Final extension	72	7 min	1
<i>cadA</i>	Initial denaturation	94	5 min	1
	Denaturation	94	1 min	
	Annealing	52	1 min	30
	Extension	72	2 min	
	Final extension	72	5 min	1

Table S4. Continuation of physicochemical properties of WR's soil samples

Site No.	EW-73	EW-85	EW-86	EW-69	EW-135
Anaerobically Mineralised N (mg kg ⁻¹)	165	31.2	24.9	222	176
Total porosity (% v/v)	64.3	60.6	62.2	58.4	60.0
Bulk density t/m ³	0.76	1.03	1.11	0.84	0.86
F *	136	340	460	340	530
Al *	36000	47000	68000	38000	59000
Sb *	0.07	0.06	0.05	0.07	0.08
As *	6.20	9.40	10.80	7.00	8.90
Ba *	240	138	114	128	166
Bi *	0.36	0.35	0.28	0.19	0.33
B *	5.00	3.00	2.00	4.00	4.00
Cs *	6.30	3.60	3.50	3.80	5.30
Ca *	2200	4600	2900	5800	5100
Cr *	13.2	18.1	23.0	56.0	19.7
Co *	10.9	3.40	4.30	10.4	12.7
Cu *	10.8	16.3	20.0	23.0	22.0
La *	5.60	7.40	8.20	12.4	9.20
Pb *	28.0	25.0	24.0	21.0	32.0
Li *	8.50	10.7	16.0	9.50	15.8
Mg *	890	470	680	1120	590
Mn *	2400	600	1080	2300	3700
Mo *	1.09	1.17	1.46	1.13	1.55
Ni *	5.20	7.10	6.00	17.70	6.70
K *	530	770	720	1380	730
Rb *	13.4	9.10	7.00	15.1	14.2
Se *	< 2.00	3.00	3.00	2.00	3.00
Ag *	0.10	0.08	0.12	0.19	0.12
Na *	250	91.0	83.0	146	120
Sr *	36.0	21.0	22.0	32.0	23.0
Ti *	0.72	0.15	0.34	0.39	0.65
Sn *	2.20	2.70	2.80	2.40	2.50
U *	1.52	2.50	2.60	2.30	2.70
V *	71.0	101	151	113	106
Hot Water C (mg Kg ⁻¹)	3359	1018	688	5010	4184
Hot Water N (mg Kg ⁻¹)	291	130	250	611	528
Hot Water C/Hot Water N	12.0	8.00	3.00	8.00	8.00
P-retension	44.0	43.0	48.0	45.0	48.0
15N	1.46	7.11	6.37	6.28	6.29

*mg kg⁻¹ of dry soil

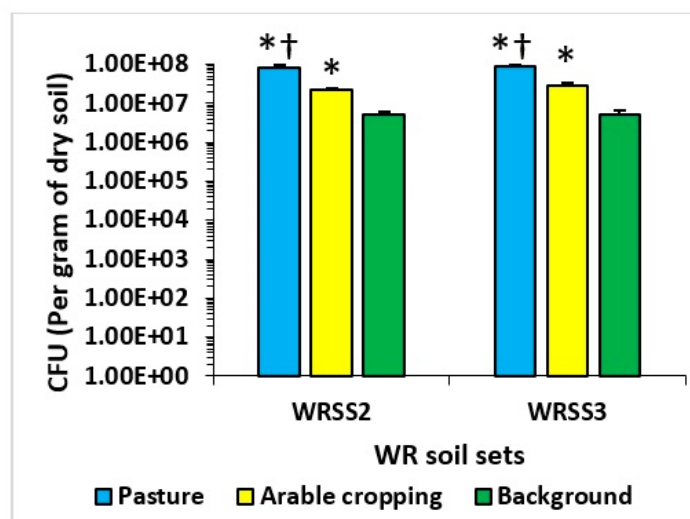


Figure S1. Total number of CFU (per gram of dry soil) from WRSS2 and WRSS3 pastoral, arable and native bush soil samples, and selected on R2A agar. * $p < 0.05$ compared to background soil bacteria total CFU; † $p < 0.05$ compared to arable soil bacteria total CFU.

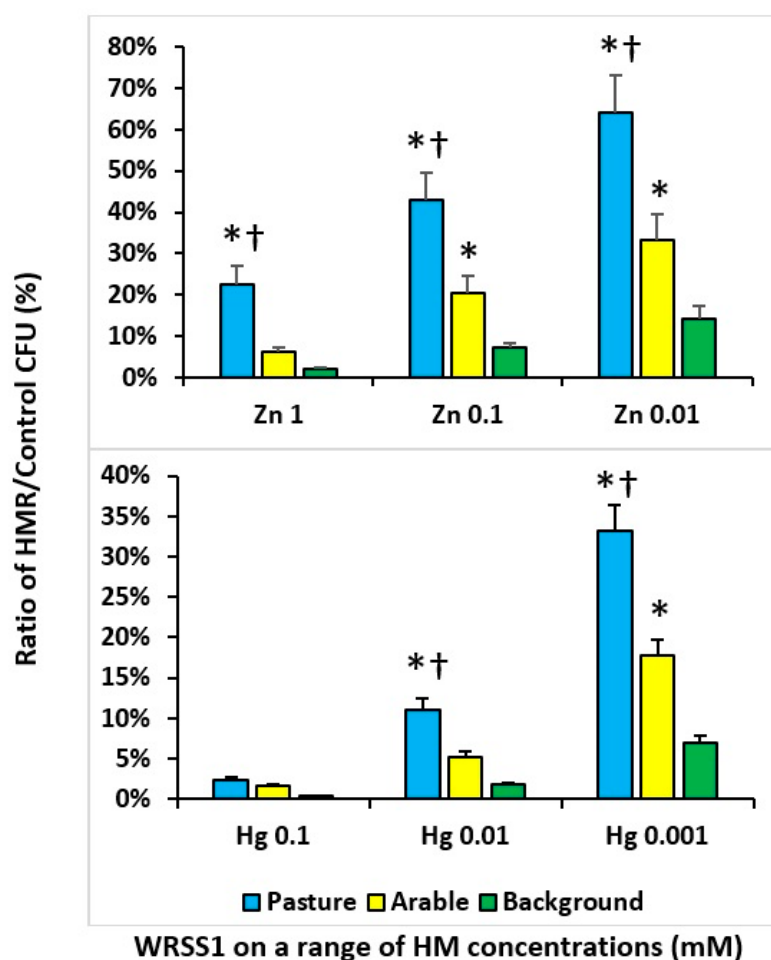


Figure S2. Mean ratios of HMR/total bacterial CFUs, on a range of Zn and Hg concentrations, for WRSS1. * $p < 0.05$ compared to background HMR/total bacterial CFU ratio selected on the same HM

concentration; †*p* < 0.05 compared to arable HMR/total bacteria CFU ratio selected on the same HM concentration.

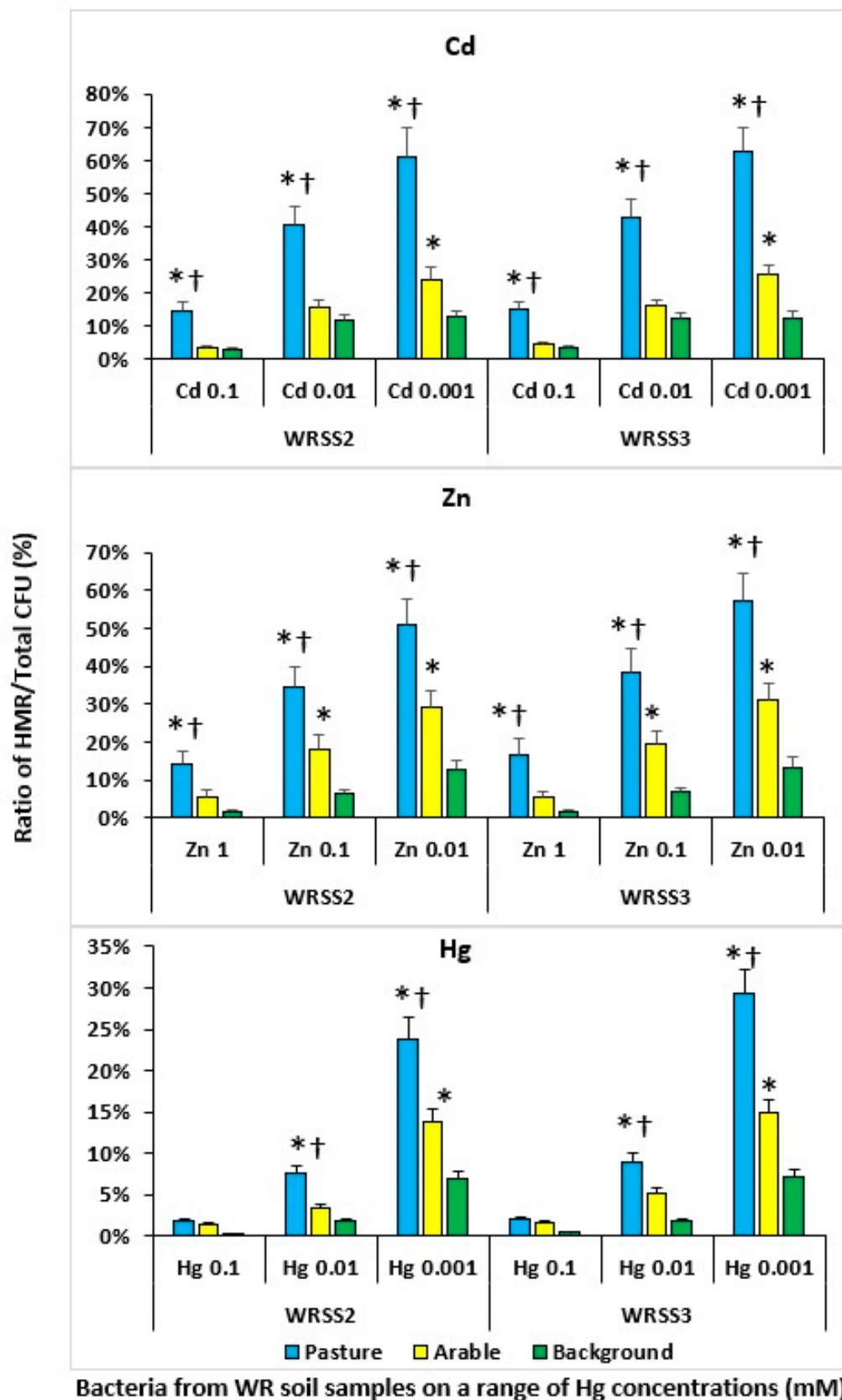


Figure S3. Mean ratios of HMR/total bacterial CFU, on a range of Cd concentrations, for WRSS2 and WRSS3. * $p < 0.05$ compared to background soil HMR/total bacterial CFU ratio selected on the same HM concentration; † $p < 0.05$ compared to arable HMR/total bacterial CFU ratio selected on the same HM concentration.

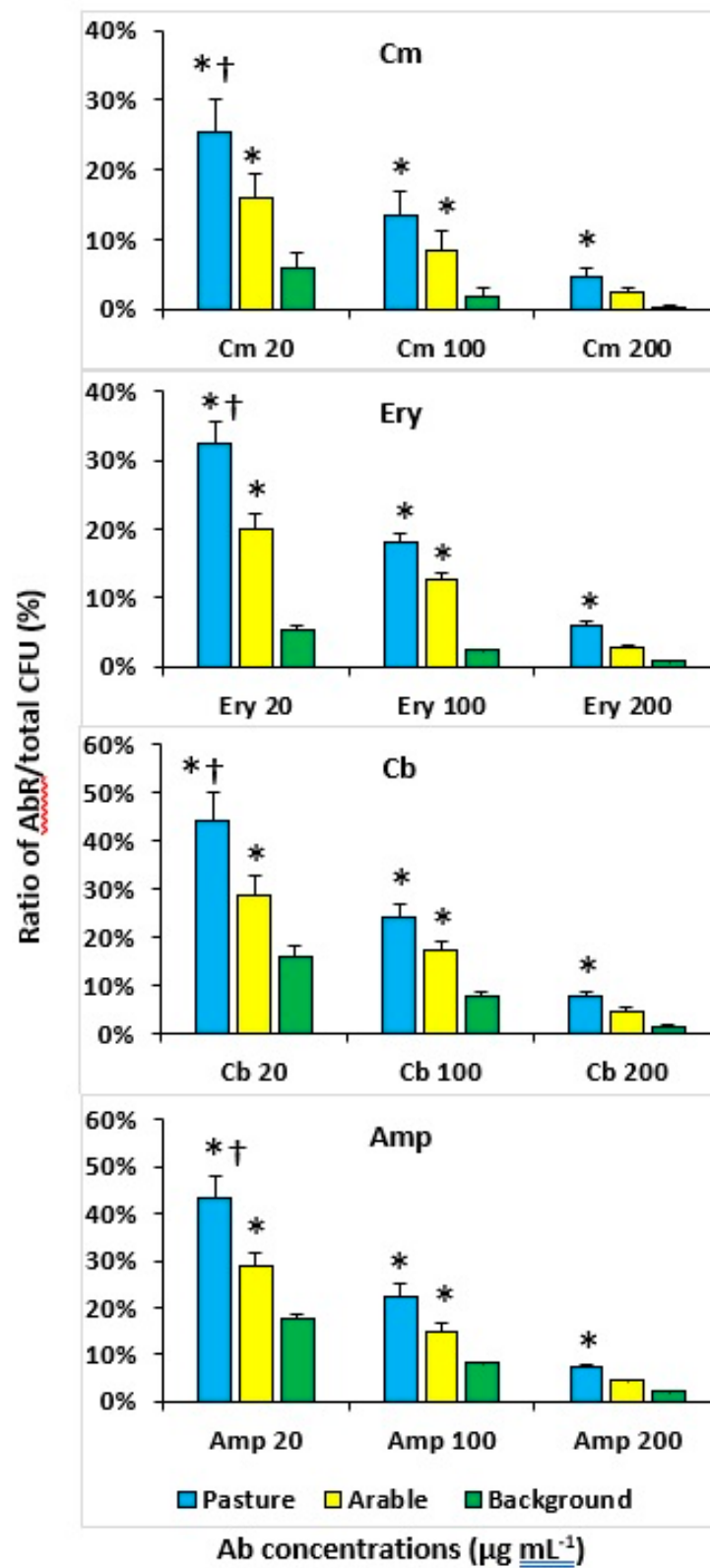


Figure S4. Mean ratios of AbR/total bacterial CFUs, on a range of Ab concentrations, for the WRSS1. $*p < 0.05$ compared to background soil AbR/total bacterial CFU ratio selected on the same Ab concentration; $\dagger p < 0.05$ compared to arable soil AbR/total bacterial CFU ratio selected on the same Ab concentration.

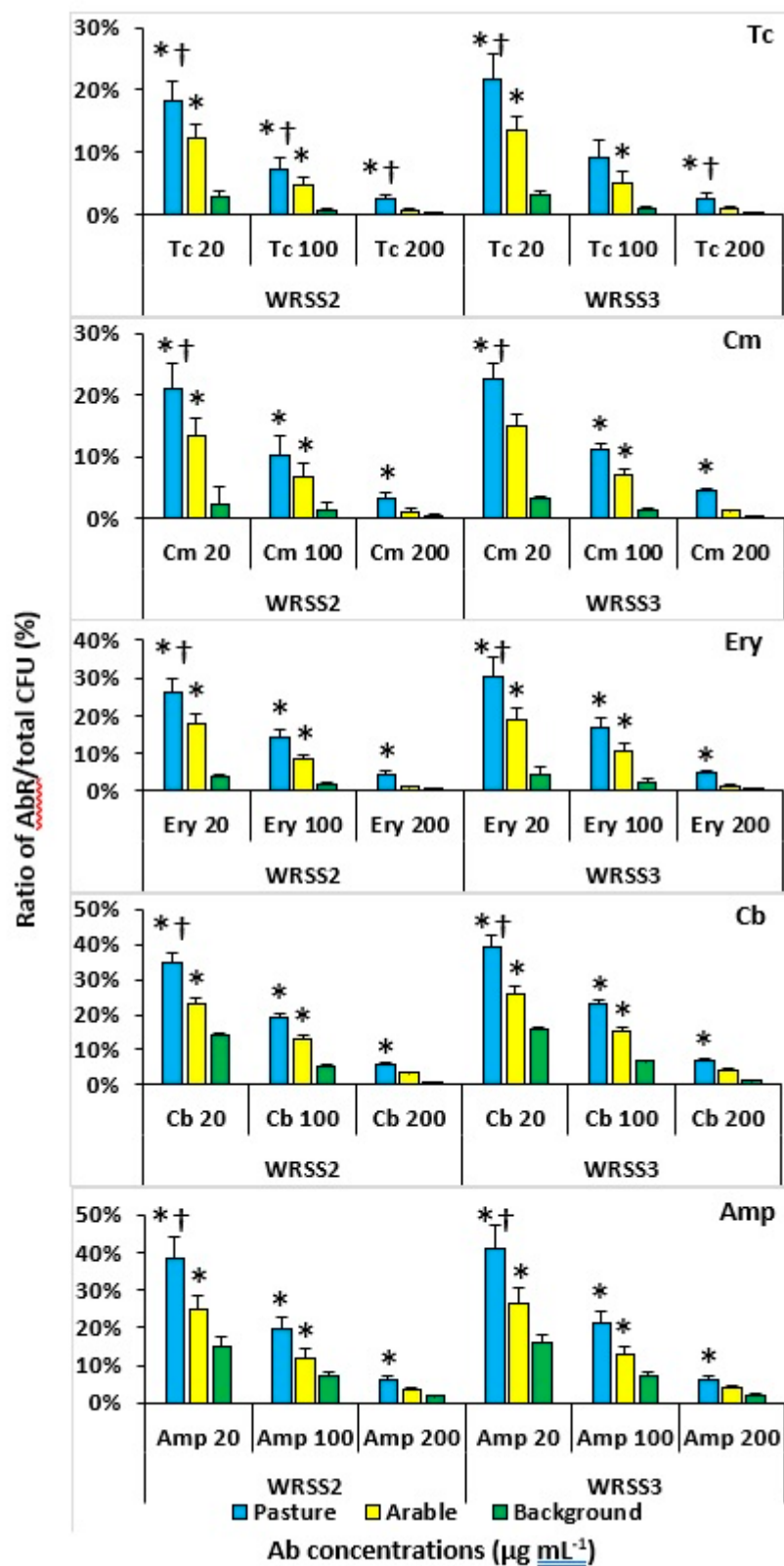


Figure S5. Mean ratios of AbR/total bacterial CFUs, on a range of Ab concentrations, for the WRSS2 and WRSS3. * $p < 0.05$ compared to background soil AbR/total bacterial CFU ratio selected on the same Ab concentration; † $p < 0.05$ compared to arable soil AbR/total bacterial CFU ratio selected on the same Ab concentration.

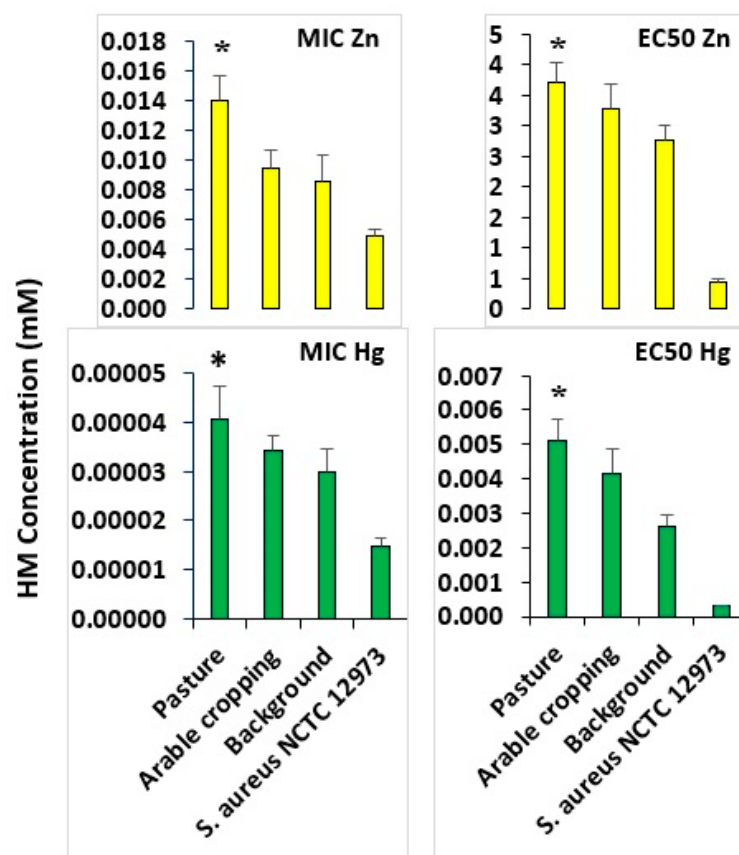


Figure S6. Mean MIC and EC50 values of PICT assay with Zn and Hg for bacteria from WRSS1. * $p < 0.05$ compared to HM MIC or EC50 values for bacteria from background soil.

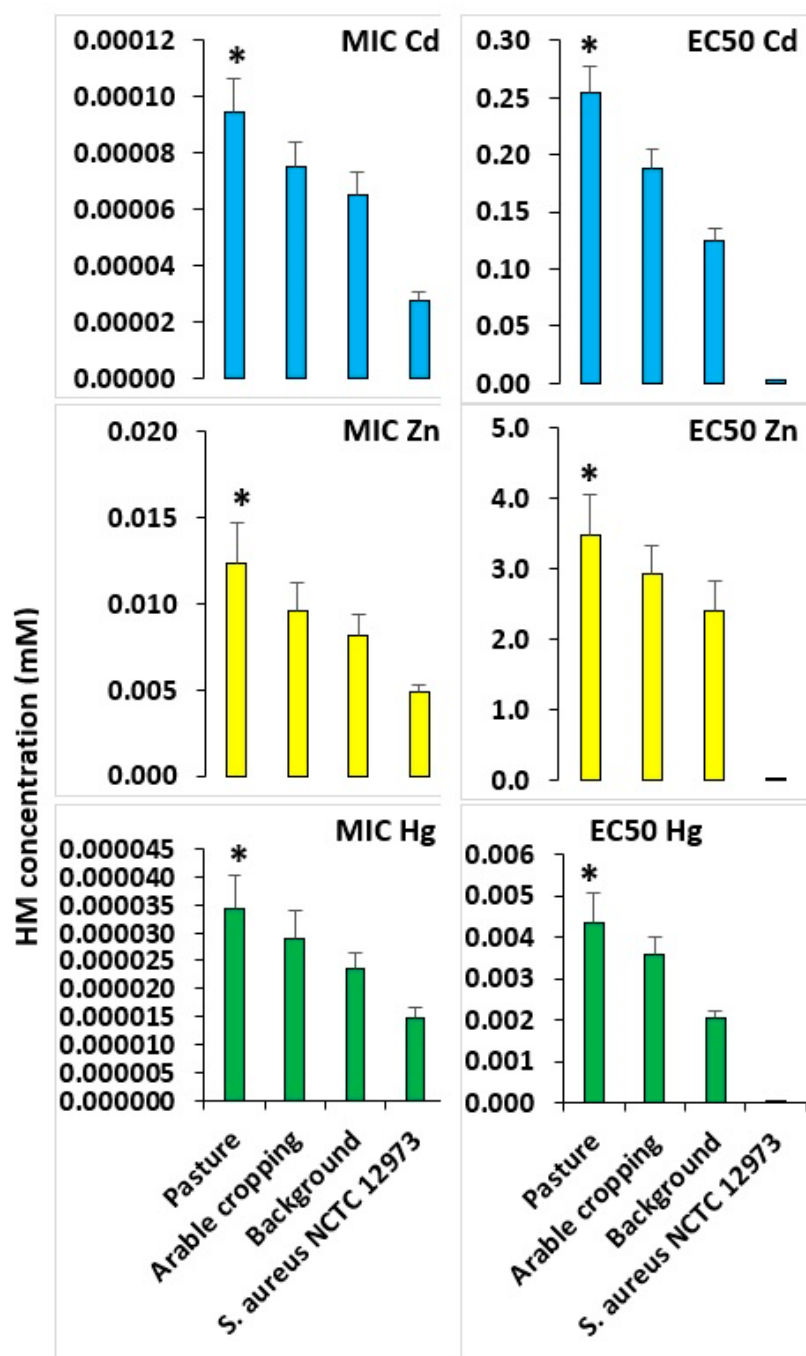


Figure S7. Mean MIC and EC50 values of PICT assay with Cd, Zn and Hg for bacteria from WRSS2. * $p < 0.05$ compared to HM MIC or EC50 values for bacteria from background soil.

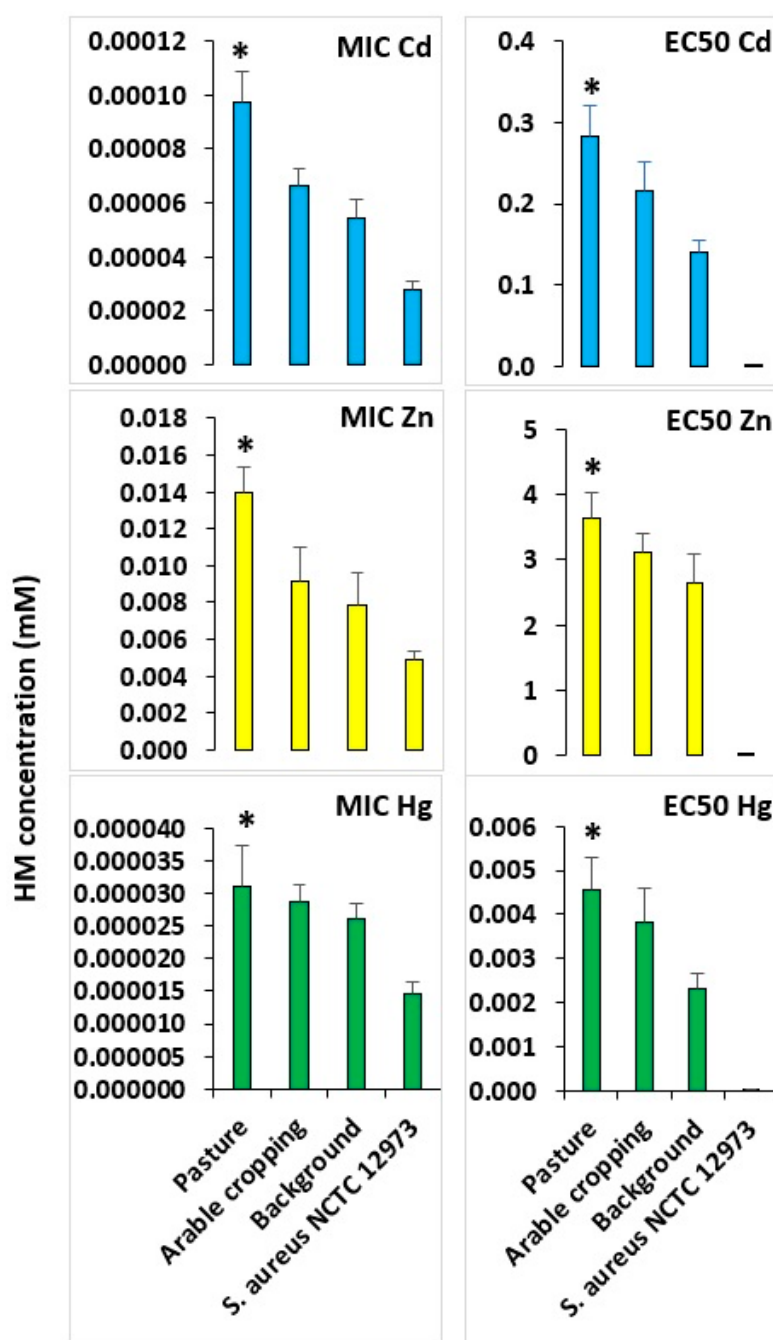


Figure S8. Mean MIC and EC50 values of PICT assay with Cd, Zn and Hg for bacteria from WRSS3. * $p < 0.05$ compared to HM MIC or EC50 values for bacteria from control soil.

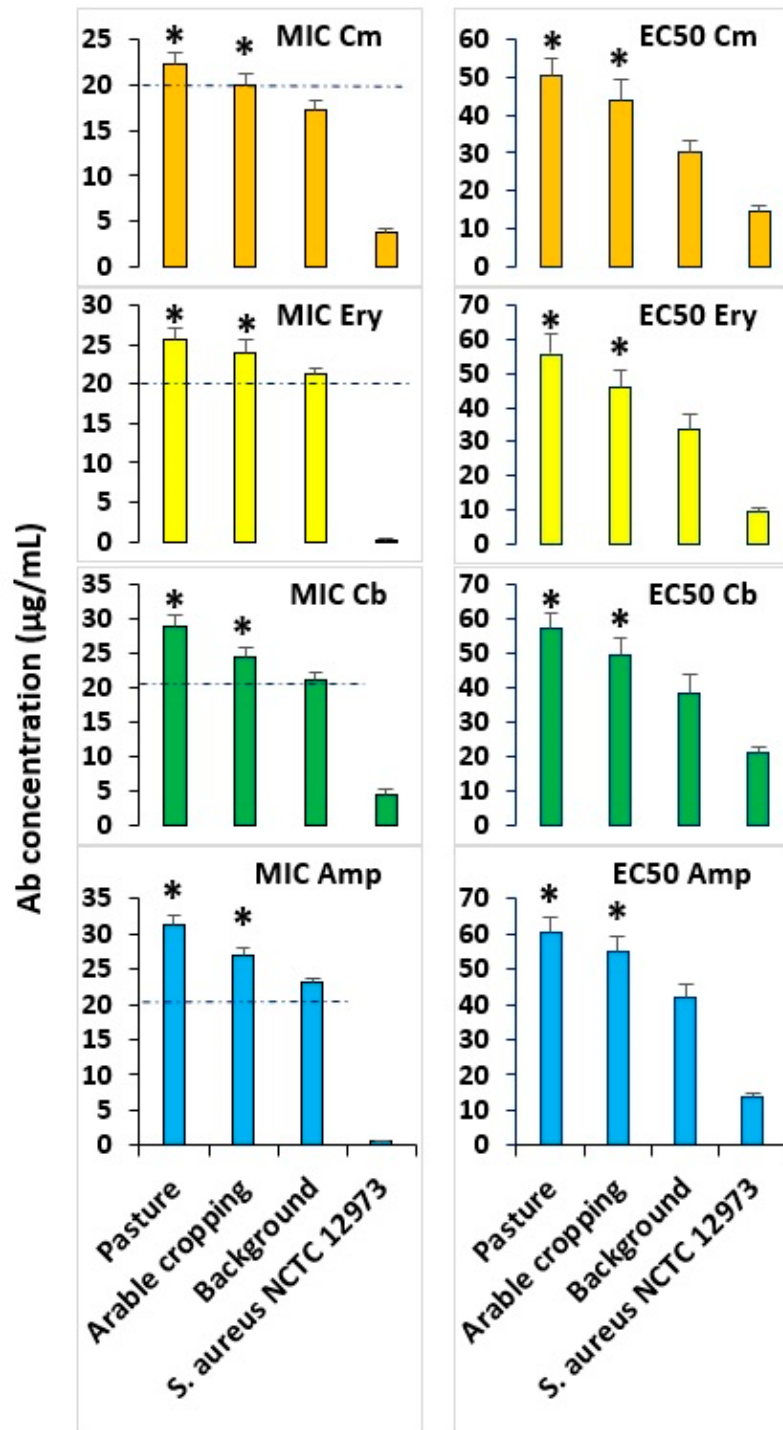


Figure S9. Mean MIC and EC50 values of PICT assay with Cm, Ery, Cb and Amp for bacteria from WRSS1. $8p < 0.05$ compared to Ab MIC or EC50 values for bacteria from background soil. The dashed line defines AbR level in soil bacteria.

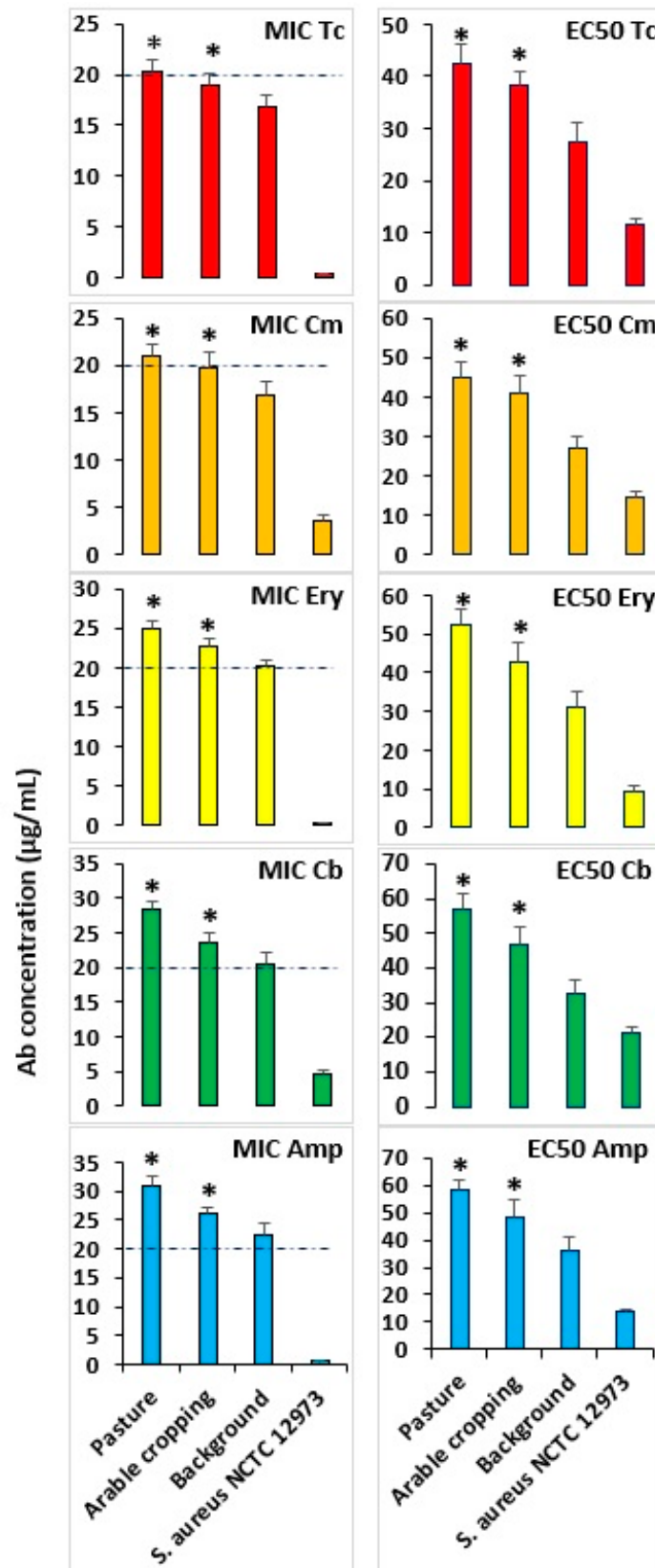


Figure S10. Mean MIC and EC50 values of PICT assay with Tc, Cm, Ery, Cb and Amp for bacteria from WRSS2. * $p < 0.05$ compared to Ab MIC or EC50 values for bacteria from control soil. The dashed line defines AbR level in soil bacteria.

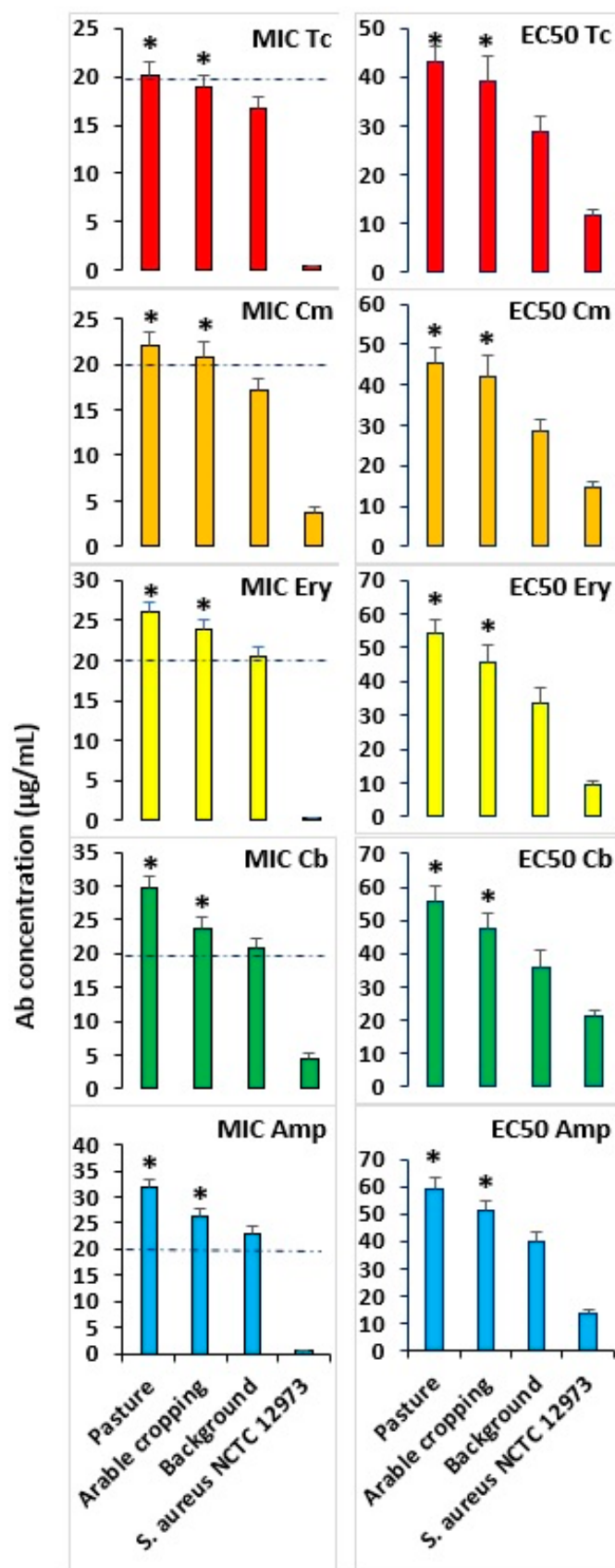


Figure S11. Mean MIC and EC50 values of PICT assay with Tc, Cm, Ery, Cb and Amp for bacteria from WRSS3. * $p < 0.05$ compared to Ab MIC values for bacteria from control soil. The dash line defines AbR level of soil bacteria.

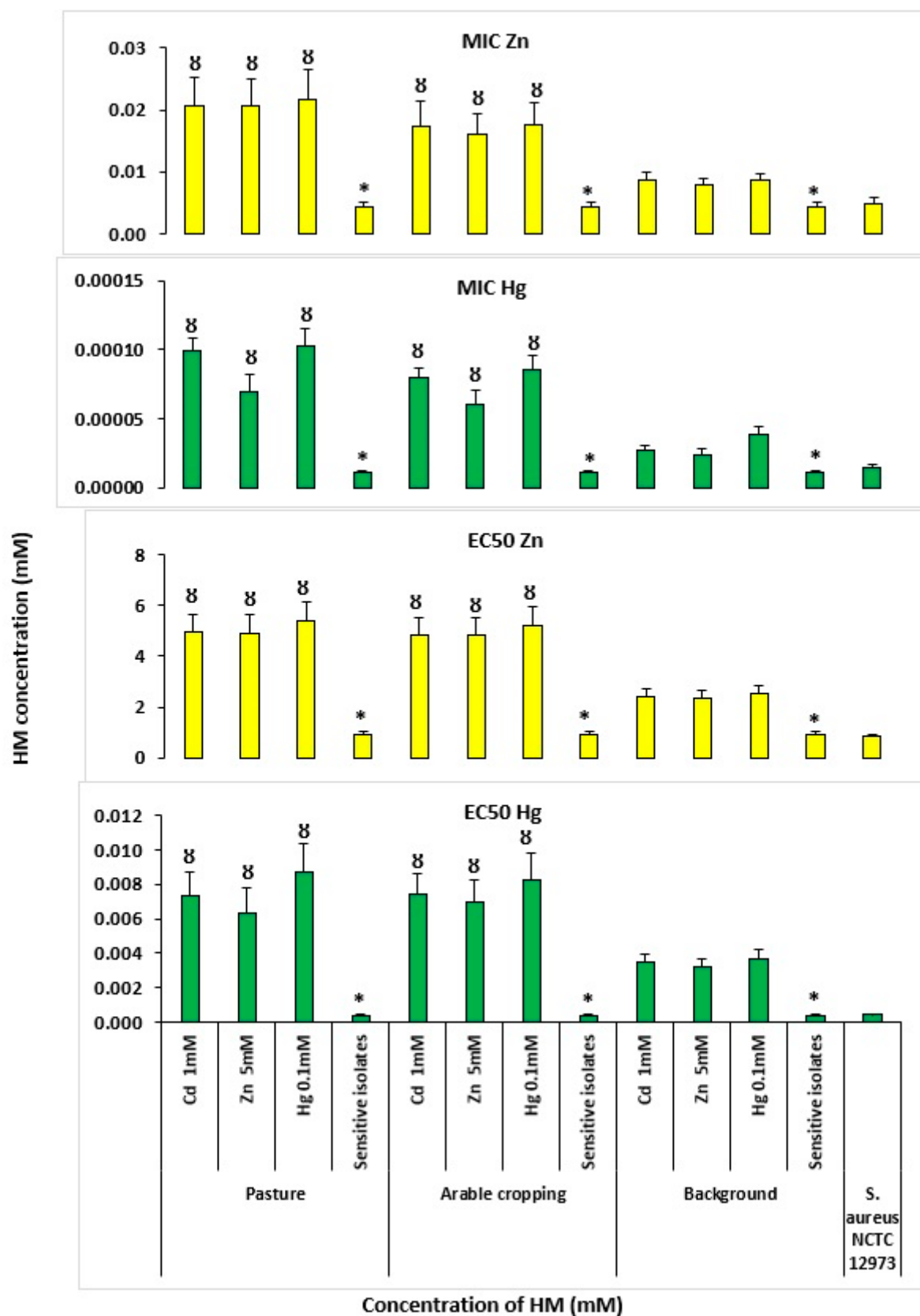


Figure S12. Mean MIC and EC50 values from Broth Microdilution assay with Zn and Hg for HMR isolates from WRSS1. * $p < 0.05$ compared to HM EC50 value for HMR isolates from the same soil; $8p < 0.05$ compared to HM EC50 value for HMR isolates from background soil.

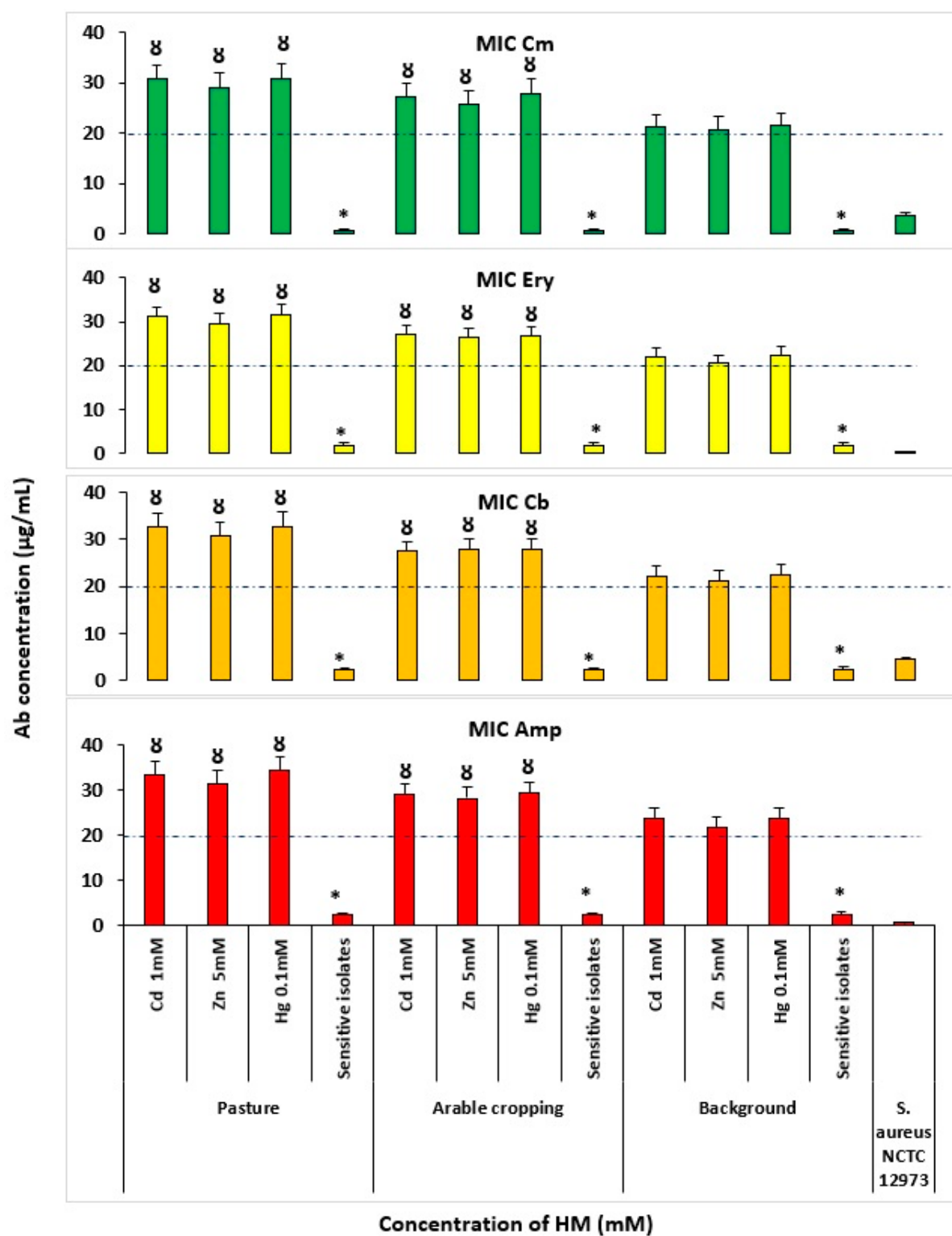


Figure S13. Mean MIC values for Broth Microdilution assay with Cm, Ery, Cb and Amp for HMR isolates from WRSS1. * $p < 0.05$ compared to Ab MIC value for HMR isolates from the same soil; $p < 0.05$ compared to Ab MIC value for isolates from background soil and selected on the same HM concentration). The dashed line defines AbR level of soil bacteria.

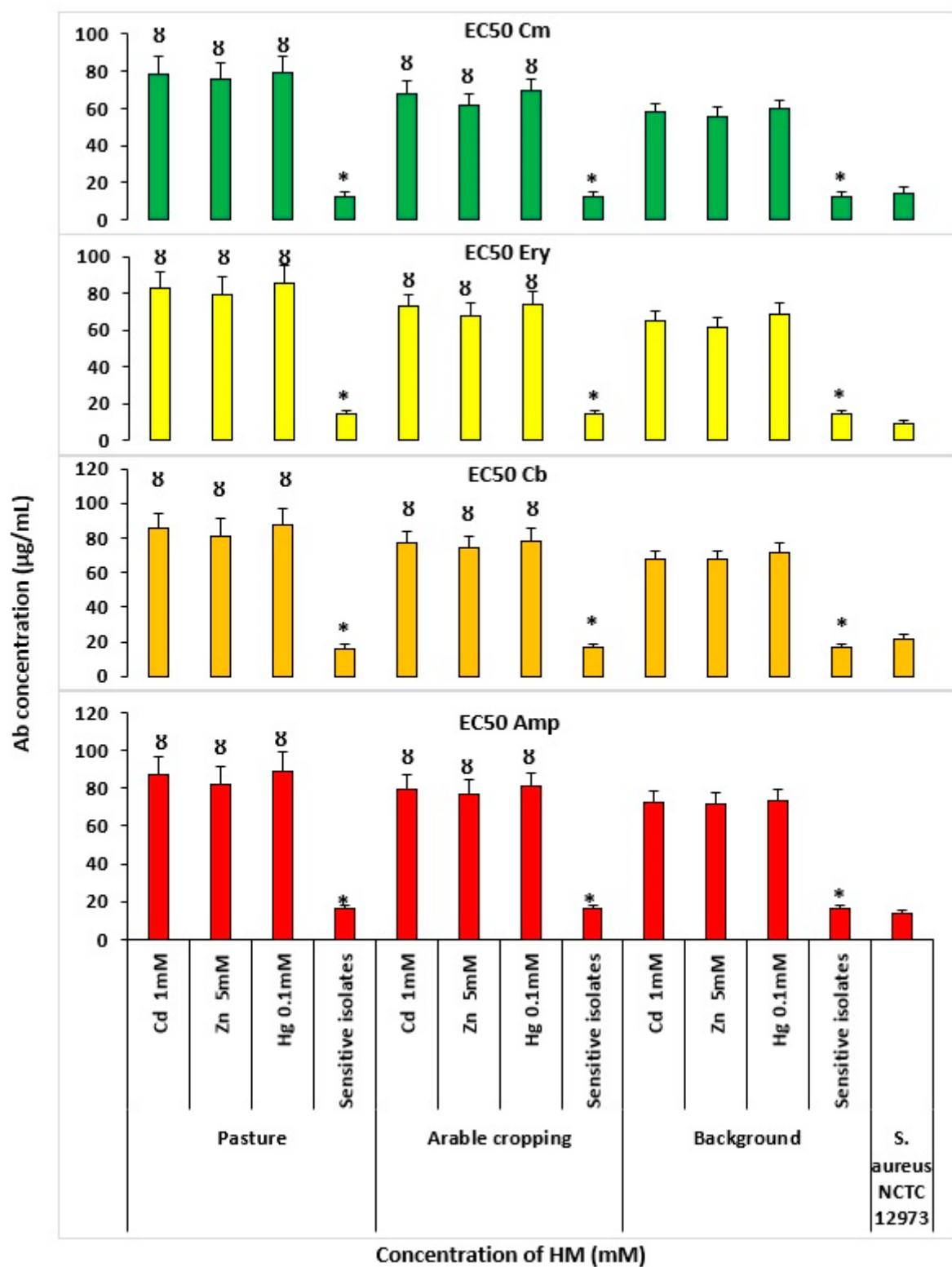


Figure S14. Mean EC50 values for Broth Microdilution assay with Cm, Ery, Cb and Amp for HMR isolates from WRSS1. * $p < 0.05$ compared to Ab EC50 value for HMR isolates from the same soil; 8 $p < 0.05$ compared to Ab EC50 value for isolates from background soil and selected on the same HM concentration.

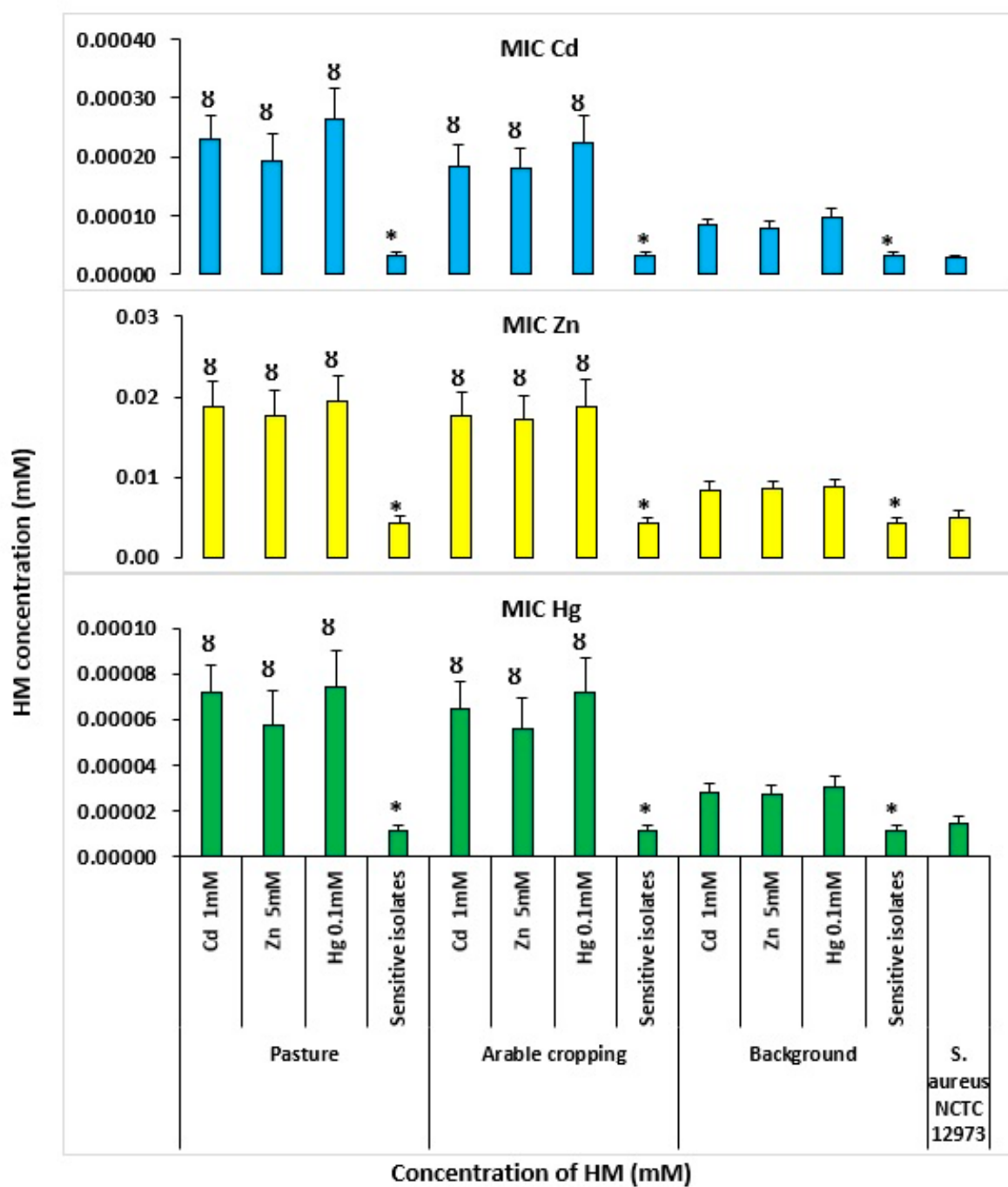


Figure S15. Mean MIC values of Broth Microdilution assay with Cd, Zn and Hg for HMR isolates from WRSS2. * $p < 0.05$ compared to HM MIC value for HM-sensitive isolates from the same soil; 8 $p < 0.05$ compared to HM MIC value for HMR isolates from control soil.

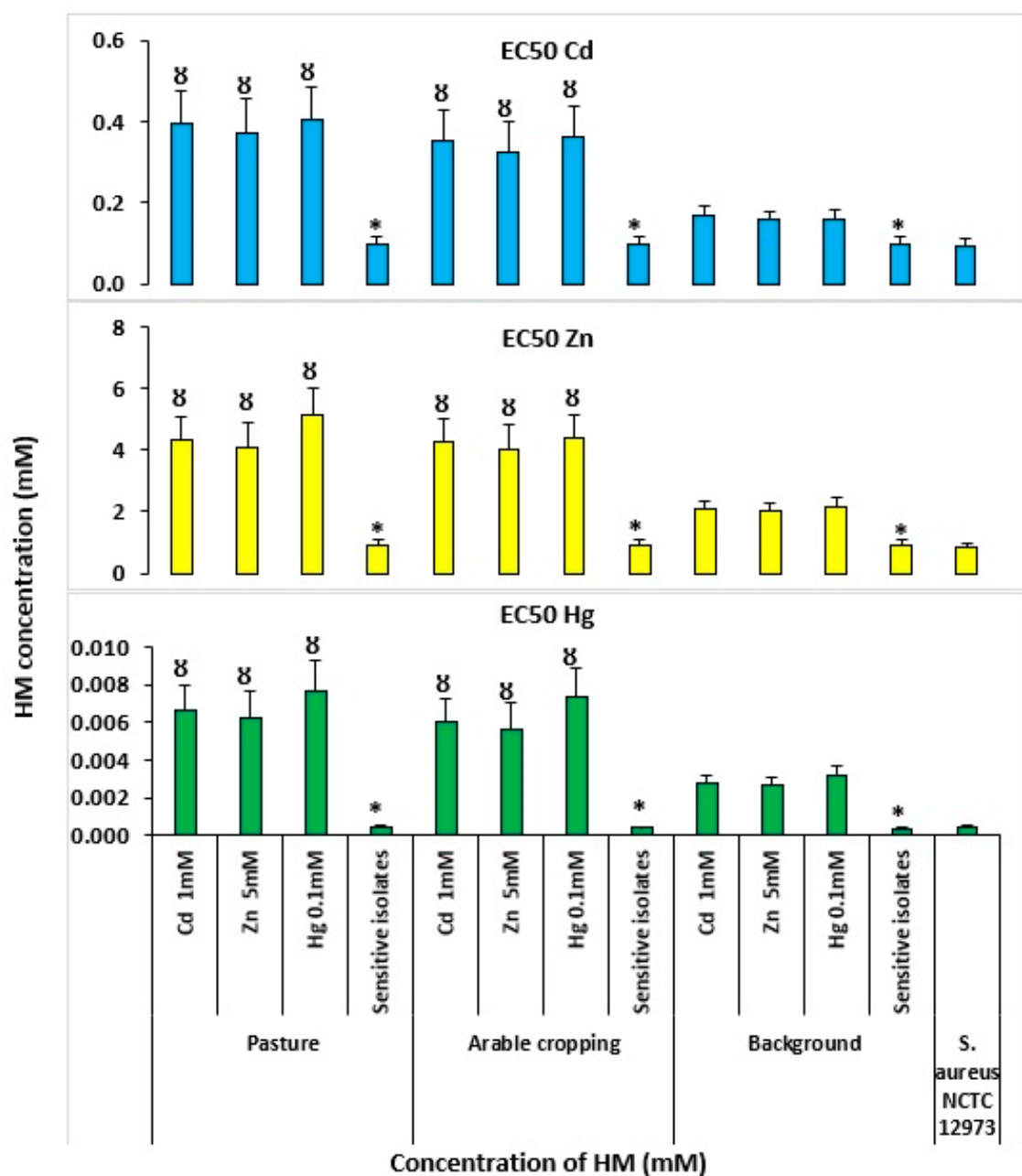


Figure S16. Mean EC50 values of Broth Microdilution assay with Cd, Zn and Hg for HMR isolates from WRSS2. * $p < 0.05$ compared to HM EC50 value for HMR isolates from the same soil; 8 $p < 0.05$ compared to HM EC50 value for HMR isolates from background soil.

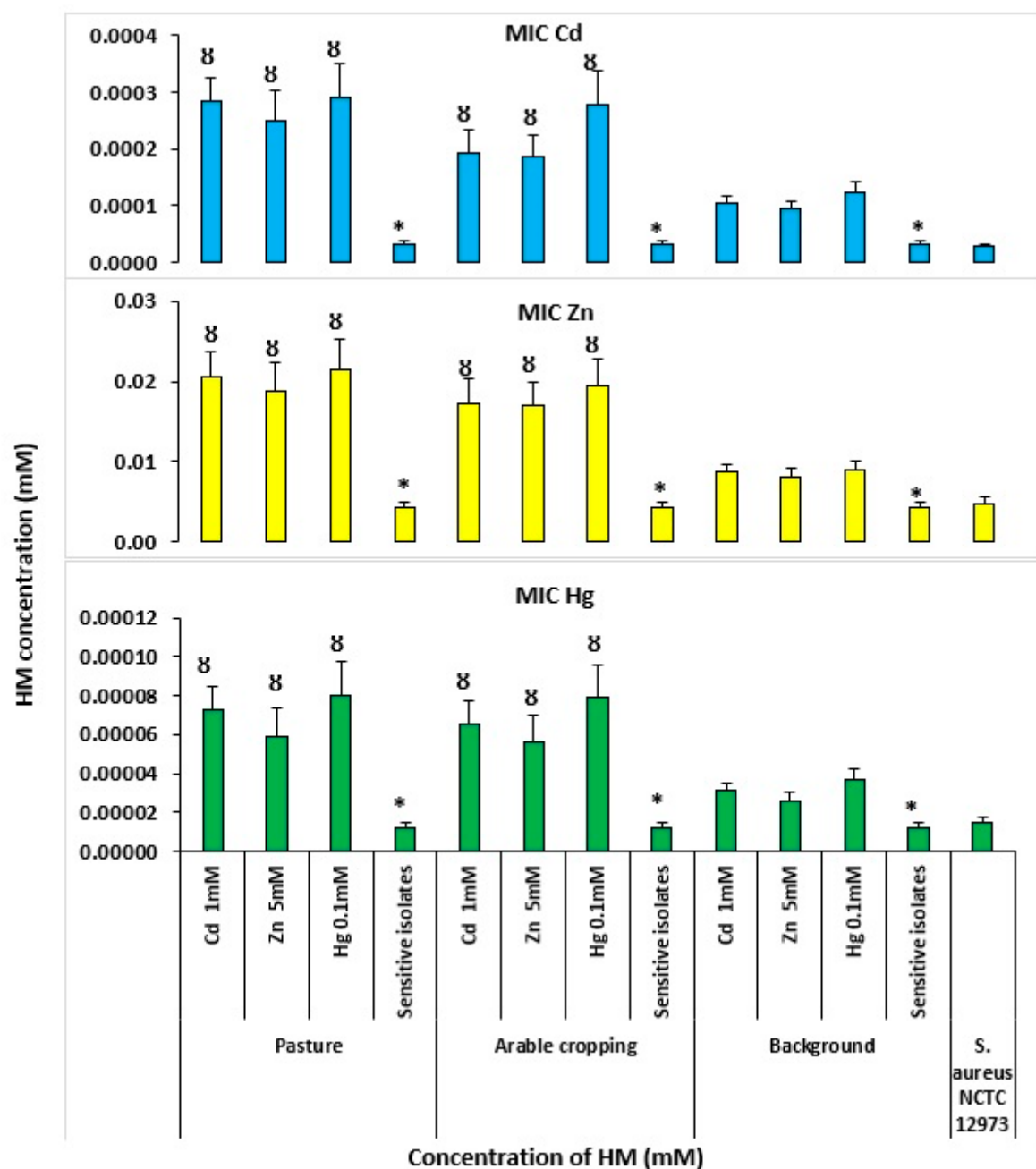


Figure S17. Mean MIC values of Broth Microdilution assay with Cd, Zn and Hg for HMR isolates from WRSS3. * $p < 0.05$ compared to HM MIC value for HM-sensitive isolates from the same soil; 8 $p < 0.05$ compared to HM MIC value for HMR isolates from control soil.

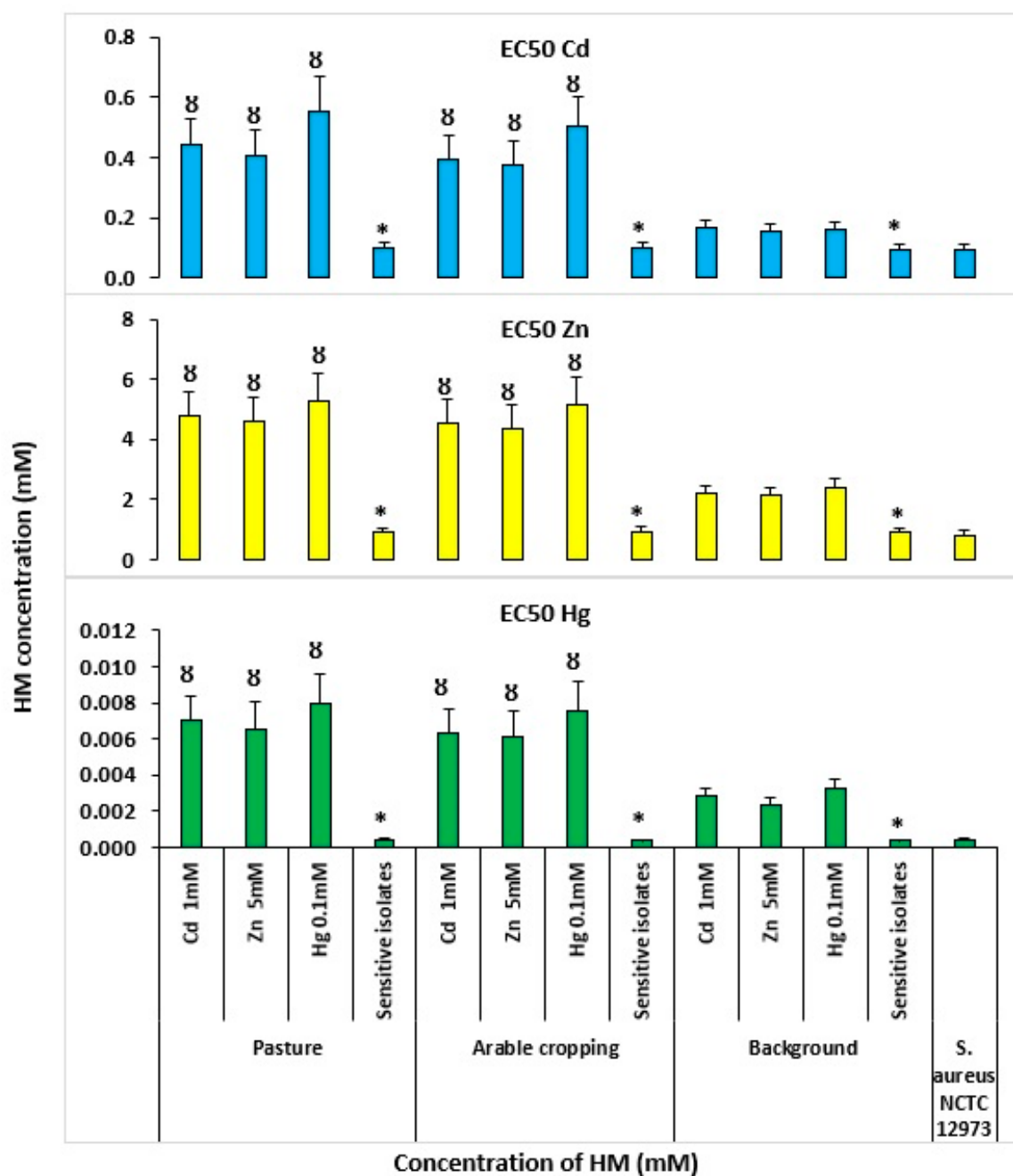


Figure S18. Mean EC50 values of Broth Microdilution assay with Cd, Zn and Hg for HMR isolates from WRSS3. * $p < 0.05$ compared to HM EC50 value for HM-sensitive isolates from the same soil; 8 $p < 0.05$ compared to HM EC50 value for HMR isolates from control soil.

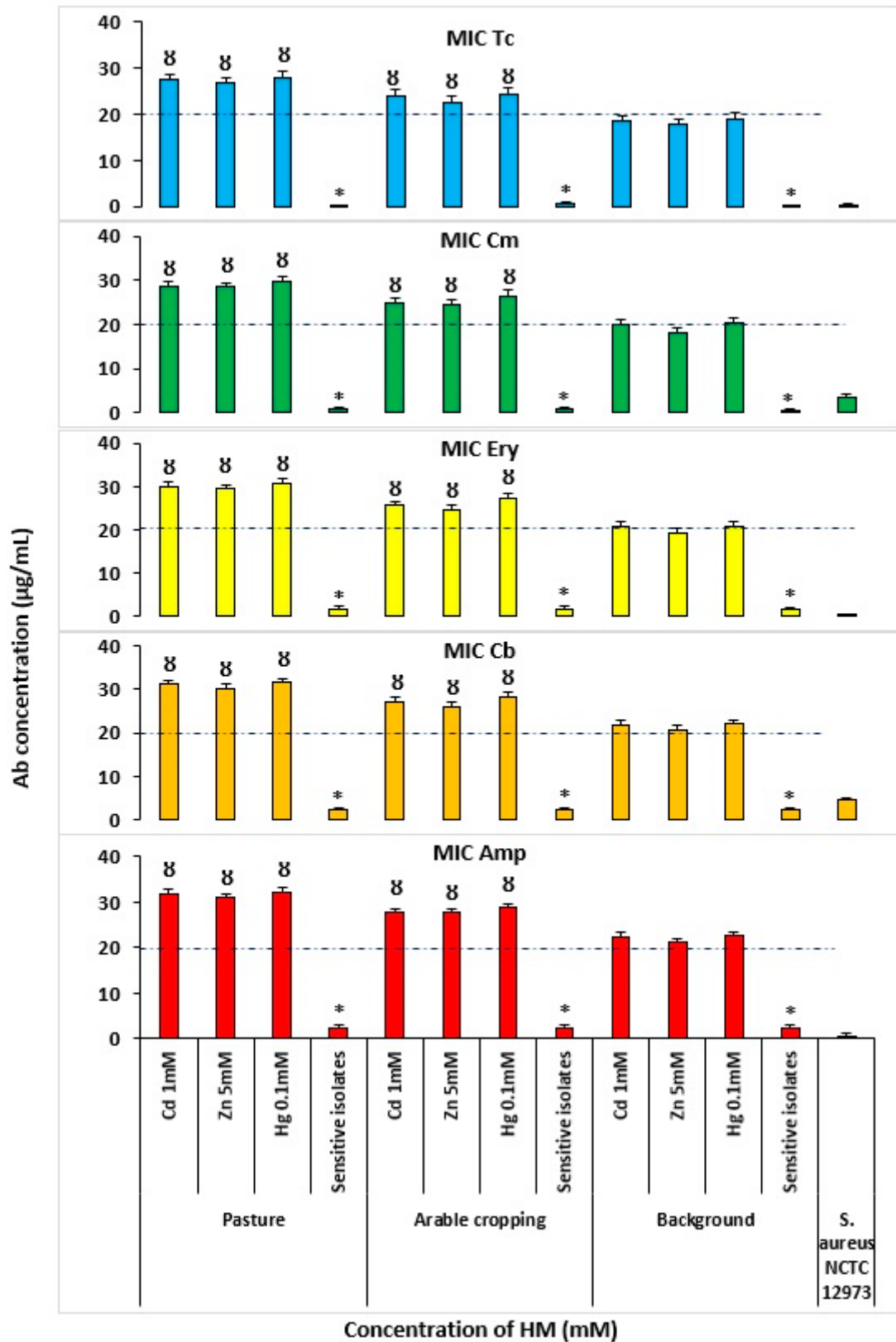


Figure S19. Mean MIC values of Broth Microdilution assay with Tc, Cm, Ery, Cb and Amp for HMR isolates from WRSS2. * $p < 0.05$ compared to Ab MIC value for HM-sensitive isolates from the same soil; 8 $p < 0.05$ compared to Ab MIC value for isolates from control soil and selected on the same HM concentration. The dashed line defines AbR level of soil bacteria.

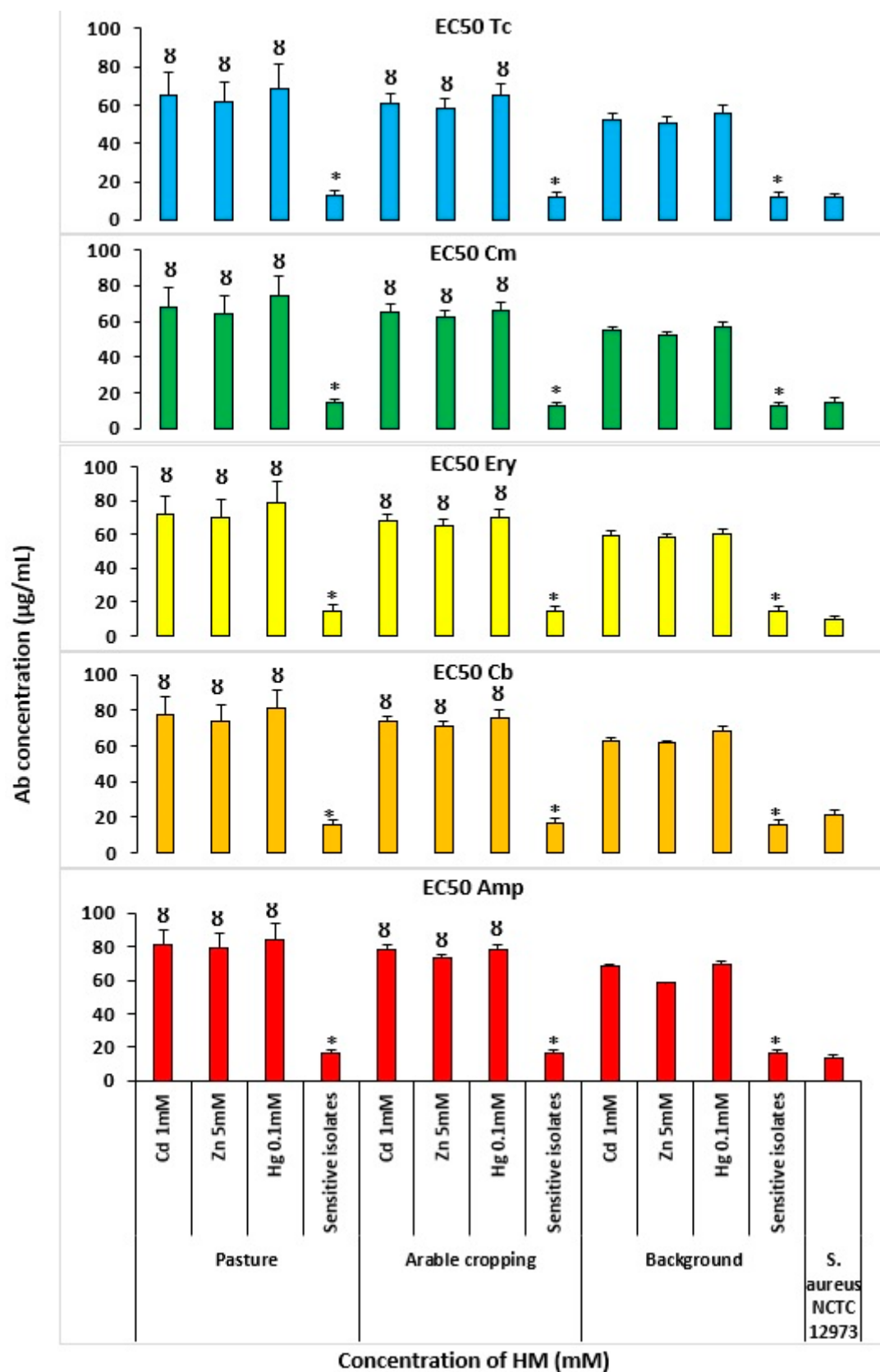


Figure S20. Mean EC50 values of Broth Microdilution assay with Tc, Cm, Ery, Cb and Amp for HMR isolates from WRSS2. * $p < 0.05$ compared to Ab EC50 value for HMR isolates from the same soil; 8 $p < 0.05$ compared to Ab EC50 value for isolates from background soil and selected on the same HM concentration.

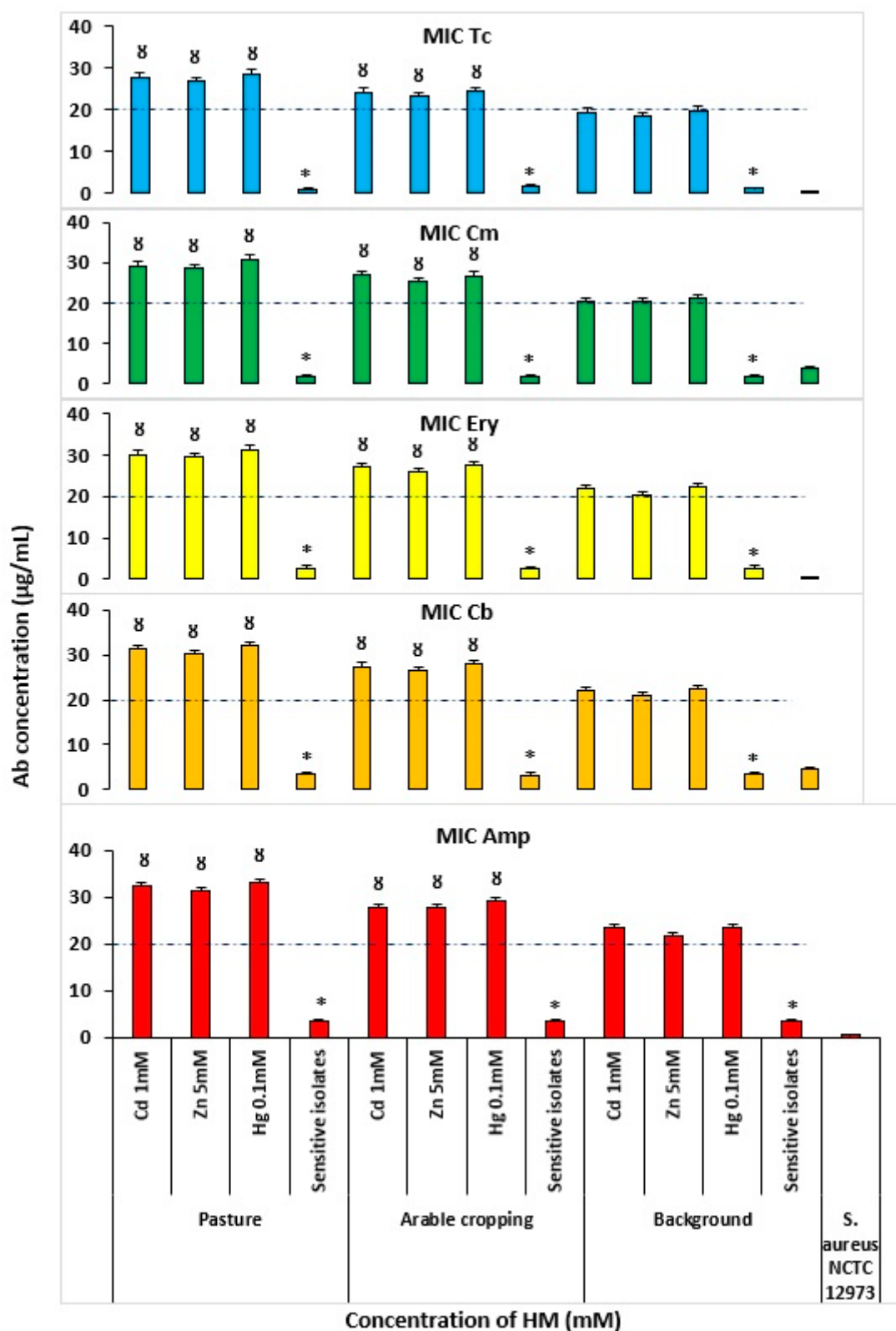


Figure S21. Mean MIC values of Broth Microdilution assay with Tc, Cm, Ery, Cb and Amp for HMR isolates from WRSS3. * $p < 0.05$ compared to Ab MIC value for HM-sensitive isolates from the same soil; 8 $p < 0.05$ compared to Ab MIC value for isolates from control soil and selected on the same HM concentration. The dashed line defines AbR level of soil bacteria.

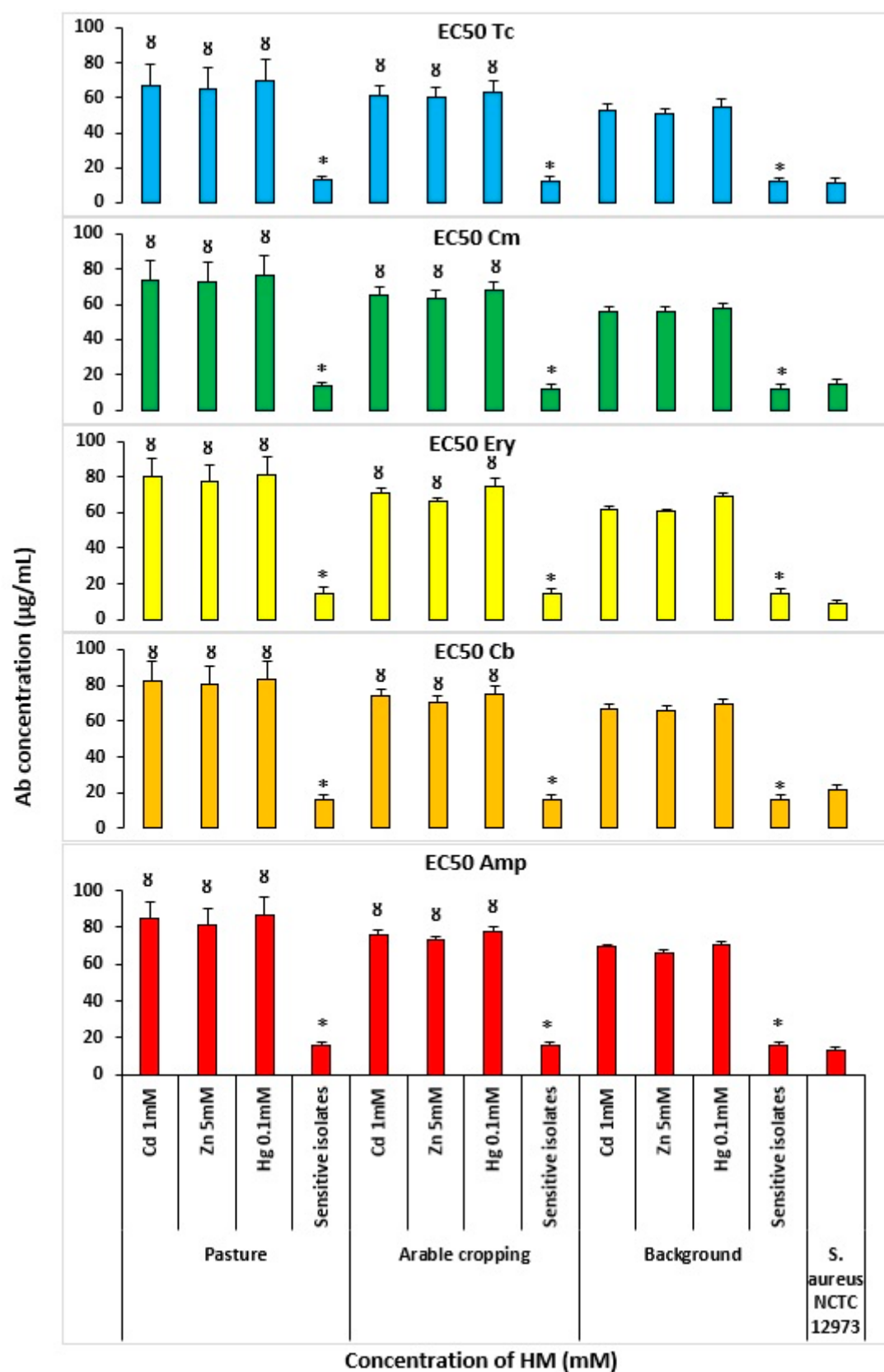


Figure S22. Mean EC50 values of Broth Microdilution assay with Tc, Cm, Ery, Cb and Amp for HMR isolates from WRSS3. * $p < 0.05$ compared to Ab EC50 value for HM-sensitive isolates from the same soil; 8 $p < 0.05$ compared to Ab EC50 value for isolates from control soil and selected on the same HM concentration.

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