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ORIGINAL PAPER

Estimation of baseline levels of bacterial community tolerance to Cr, Ni, Pb, and Zn in unpolluted soils, a background for PICT (pollution-induced community tolerance) determination

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Abstract
The PICT method (pollution-induced community tolerance) can be used to assess whether changes in soil microbial response are due to heavy metal toxicity or not. Microbial community tolerance baseline levels can, however, also change due to variations in soil physicochemical properties. Thirty soil samples (0–20 cm), with geochemical baseline concentrations (GBCs) of heavy metals and from five different parent materials (granite, limestone, schist, amphibolite, and serpentine), were used to estimate baseline levels of bacterial community tolerance to Cr, Ni, Pb, and Zn using the leucine incorporation method. General equations ($n = 30$) were determined by multiple linear regression using general soil properties and parent material as binary variables, explaining 38% of the variance in log IC_{50} (concentration that inhibits 50% of bacterial growth) values for Zn, with 36% for Pb, 44% for Cr, and 68% for Ni. The use of individual equations for each parent material increased the explained variance for all heavy metals, but the presence of a low number of samples ($n = 6$) lead to low robustness. Generally, clay content and dissolved organic C (DOC) were the main variables explaining bacterial community tolerance for the tested heavy metals. Our results suggest that these equations may permit applying the PICT method with Zn and Pb when there are no reference soils, while more data are needed before using this concept for Ni and Cr.

Keywords Bacterial growth · Leucine incorporation · Heavy metals · Geochemical baseline level · Co-tolerance

Introduction

Heavy metals occur naturally in soils, but they can also appear in the environment as a result of anthropogenic activities, such as mining or smelting, or through the application of sewage sludge, inorganic fertilizers, or livestock manure (Alloway 2012). The assessment of the impacts derived from heavy metal pollution in soils remains a challenge, since direct methods used for metal impact determinations are based on the extraction of different metal fractions, e.g., soluble, exchangeable, bioavailable, or total content (Peijnenburg and Jager 2003; Rauret 1998; Senwo and Tazisong 2004). Such methods do not necessarily take into account the damage exerted on the soil biota.

Microorganisms are important endpoints to determine the effects of heavy metal pollution because of their sensitivity and important functions in soil (Imfeld et al. 2011; Nannipieri et al. 2003). It is well known that soil microbial communities can be negatively affected by heavy metals (e.g., Anderson et al. 2009; Bååth 1989; Bérard et al. 2016; de Lima e Silva et al. 2012; Giller et al. 1998; Stefanowicz et al. 2009, 2010; Vázquez-Blanco et al. 2020, 2021; Wang et al. 2010; Zhou et al. 2009), and hence their essential role in maintaining soil functions can be altered. Therefore, the use of soil microbial communities as bioindicators constitutes a good alternative to chemical methods to assess if soil functions are being affected by heavy metals (Puglisi et al. 2012).

To study the effect of heavy metals on soil microorganisms, methods based on soil respiration, nutrient use efficiency, microbial biomass, microbial activity, community composition, etc., are commonly used (Anderson et al. 2009;

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Bérard et al. 2016; Boivin et al. 2006; Macdonald et al. 2010; Niklińska et al. 2006; Salminen et al. 2001; Stefanowicz et al. 2010). However, these methods are only reliable under laboratory conditions or with a single soil with appropriate unpolluted control. In field studies, where soil properties may be highly different among different soil types, it will be difficult to distinguish if observed changes are due to metal toxicity or variation in soil properties (Niklińska et al. 2005). For example, Stefanowicz et al. (2010) reported that in metal-polluted soils, microbial performance was mainly determined by soil physicochemical properties. Furthermore, some studies suggest that soil properties can affect the microbial response more than the metal content does (Kamitani et al. 2006). Joynt et al. (2006) showed that changes in microbial biomass from Cr, Pb, and petroleum polluted soils were related to the total amount of organic C and N in the soil, whereas Cr or Pb concentrations were not significant for predicting community changes.

A solution, to deal with the issue of the influence of soil properties, is to use methods directly related to heavy metal toxicity such as the pollution-induced community tolerance (PICT) method proposed by Blanck et al. (1988); a methodology that can be used with different types of microbial communities (Bérard et al. 2016; Boivin et al. 2006; Stefanowicz et al. 2009; Van Beelen et al. 2004). The concept of PICT consists of two phases: a selection phase, where the microbial communities are exposed to the toxicant in the field or in the laboratory, and a detection phase, in which tolerance measurements are obtained by a second exposure of the microorganisms to the toxicant in a short-term laboratory assay. In the PICT method, it is assumed that microorganisms have different sensitivity to a pollutant and, when the pollutant is present in high enough concentrations, sensitive species will be replaced by more tolerant ones. Thus, the total microbial community will increase its tolerance to the toxic pollutant, which could be detected by determining community tolerance to the pollutant in the short-term assay.

The PICT method can be used with different endpoints in the detection phase, such as different activities of the soil bacterial communities (Blanck 2002; Tlili et al. 2016). Since bacterial growth is very susceptible to toxicants, it is considered a sensitive stress indicator (Bååth 1992). The leucine incorporation technique (as a bacterial growth proxy) is a well-established, highly sensitive, and economical assay that allows a large number of samples to be processed (Bååth et al. 2001; Brandt et al. 2009; Imfeld et al. 2011). The leucine incorporation technique has previously been applied satisfactorily in agricultural, industrial, and forest soils to perform PICT assays with heavy metals (Bååth et al. 2005; Díaz-Raviña and Bååth 1996; Santás-Miguel et al. 2020; Shi et al. 2002) or other pollutants (Brandt et al. 2009; Demoling and Bååth 2008; Demoling et al. 2009; Liu et al. 2012; Schmitt et al. 2004, 2006).

However, the PICT method can present some difficulties in detecting metal pollution. For example, Boivin et al. (2006) tried to discriminate the metal effect (Pb, Zn, Cu, and Cd) on bacterial communities in soils with different properties but did not find a relationship between metal concentrations and tolerance to those metals despite large concentrations. Stefanowicz et al. (2009) found similar bacterial tolerance values in polluted forest humus when compared to control soils. Other authors found different tolerance values for the same level of Cu and Zn as a function of applied treatment (sludge cake, liquid sludge, metal salts), which suggest that soil type had a predominant influence on tolerance development (Macdonald et al. 2010). Wang et al. (2010) found tolerant microorganisms to Cd in unpolluted soils, indicating that tolerance development to Cd was due to another cause than Cd pollution. All this suggests that in non-polluted soils with different soil properties, bacterial community tolerance to heavy metals may differ. These changes may be due to two different causes. First, heavy metal toxicity or bioavailability varies in soils with different general properties despite the similar total concentration of metals. This will affect the results during the selection phase of PICT when bacteria are exposed to heavy metals. Secondly, differences in tolerance measurements may be due to possible artifacts during the detection phase of PICT. For example, Lekfeldt et al. (2014) obtained very different values of bacterial community tolerance to Cu in unpolluted soils with different amendments, suggesting artifacts during measurements (such as pH changes or different concentrations of dissolved organic matter in the bacterial suspensions). Bérard et al. (2016) studied the long-term impact of Pb on soil microorganisms and showed that soil organic C can interfere with the microbial response since the presence of organic C can reduce Pb bioavailability, overestimating tolerance measurements.

To find a non-polluted control soil with similar properties as the soil studied, to be used as a reference, is not always possible. Therefore, it would be desirable to obtain bacterial community tolerance to heavy metal baseline levels as a function of soil properties. In a previous study, we found that in unpolluted soils with geochemical baseline concentrations (GBCs) of Cu, bacterial community tolerance to Cu baseline levels could be predicted using soil properties such as dissolved organic C (DOC), soil pH, clay, or organic matter content (Campillo-Cora et al. 2021). In order to study soils polluted with other heavy metals, similar studies are needed. We hypothesized that baseline levels of bacterial community tolerance to other heavy metals (Cr, Ni, Pb, and Zn) can be also predicted using soil properties similar to those found previously for Cu, since these properties also affect Cr, Ni, Pb, and Zn in a similar way, although the magnitude of the effects may be different (Alloway 2012). We further hypothesized that parent material also affects bacterial community tolerance to heavy metal determinations. The aim of the present study was to estimate the baseline levels of bacterial community tolerance to Cr, Ni, Pb, and Zn as a function of soil properties. The PICT methodology and the leucine incorporation technique were used to determine the bacterial community tolerance to these heavy metals in soils with GBCs, developed over different soil parent materials and with a wide range of physicochemical properties.

Materials and methods

Soil samples

The soil samples were the same used previously by Campillo-Cora et al. (2021) to estimate the bacterial community tolerance to Cu baseline levels. In brief, 30 isolated forest soils in Galicia (NW Spain), with no human activity, were selected for sampling. These unpolluted soils were considered to have GBCs of the studied metals (Cr, Ni, Pb, and Zn). The soils were developed from five parent materials: granite, limestone, schist, amphibolite, and serpentine, thus ensuring a wide range of general soil properties. From each parent material area (selected using geological maps), sampling locations were selected randomly (Figure S1; Supplementary Information). At each location, 8–10 subsamples (0 to 20 cm depth) were taken using an Edelman probe and mixing them into one composite sample. If an organic layer was present, it was removed before soil sampling. For each parent material, six sites were sampled, that is thirty sites in total. The samples were air-dried, homogenized, sieved (2-mm mesh size), and stored until analyses.

Soil properties

Detailed descriptions of the chemical analyses are given in Campillo-Cora et al. (2021). Soil texture was determined by the international pipette method (Day 1965; Green 1981). The percentage of organic matter (%OM) was measured by calcination (550 °C, 3 h) (Hoogsteen et al. 2015). A ratio of 1:5 soil/water was used to extract DOC (Jones and Willett 2006) and was measured using an analyzer multi N/C 2100 (Analytik Jena, Germany). Soil pH was measured in water (pH_w) and in 0.1 M KCl (pH_K) with a glass electrode. Effective cation exchange capacity (eCEC) was calculated as the sum of exchangeable base cations extracted with 0.2 M NH_4Cl and exchangeable aluminum extracted with 1 M KCl (Bertsch and Bloom 1996; Sumner and Miller 1996), all ions were measured by atomic absorption spectroscopy (AAS) using a Thermo Solaar Spectrometer (Thermo, USA). To extract (Fe, Al)-compounds associated with organic matter (Fe_{om}, Al_{om}), Na-pyrophosphate was used (Bascomb 1968) and ammonium oxalate-oxalic acid to obtain inorganic noncrystalline oxyhydroxides and metal-humus complexes (Fe_{ox}, Al_{ox}) (Blakemore 1978). Total free Fe (Fe_t) was extracted with Na-dithionite-citrate (Holmgren 1967), and total free Al (Al_t) was extracted with NaOH. All (Fe, Al)-extracts were measured by AAS using a Thermo Solaar Spectrometer. Inorganic amorphous Fe and Al (Fe_{ia}, Al_{ia}) were calculated by the difference between $(Fe, Al)_{ox}$ and $(Fe, Al)_{om}$. To determine Fe and Al bound to crystalline structures (Fe_c, Al_c), the difference between $(Fe, Al)_t$ and $(Fe, Al)_{ox}$ was calculated. The total amount of metals (Cr, Cu, Ni, Pb, and Zn) was obtained by soil digestions with HNO_3, HF, and HCl (Reed and Martens 1996) in a MarsXpress microwave oven (CEM Corporation, USA) and measuring heavy metals by AAS using a Thermo Solaar Spectrometer. In addition, available P by the Bray-II method and total C and N were also measured using a Thermo Finnigan EA 1112 elemental analyzer (Thermo, USA). Bioavailable heavy metals in soils were determined through 0.02 M EDTA-Na_2 extraction (Lakanen and Erviö 1971), except for limestone soils in which extractions were performed with 0.005 M DTPA + 0.1 M TEA (Lindsay and Norwell 1978) due to the high pH of these soils.

The properties of all soils (30) can be found in Tables S1–S4 (Supplementary Information). In brief, soil samples presented a wide range of particle size distribution (19–71% sand, 13–67% silt, and 13–32% clay). Soil pH measured in water (pH_w) varied between 3.8 and 7.8. and pH measured in KCl between 2.7 and 7.4. A range from 2 up to 32 $cmol_c \cdot kg^{-1}$ was obtained for eCEC. A large variation was determined for DOC and OM contents: 0.14–1.46 $g \cdot kg^{-1}$ and 7–29%, respectively. The general average abundance of Fe fractions followed the order: $Fe_c > Fe_{om} > Fe_{ia}$. For Al, the general average sequence was $Al_{om} > Al_c > Al_{ia}$. It should be noted that both sequences could differ as a function of the parent material. Total Pb, Cu, and Zn presented low values: 7–84, 4–106, and 36–203 $mg \cdot kg^{-1}$. For Cr and Ni, a higher variation was found (7–4886 and 5–2322 $mg \cdot kg^{-1}$, respectively). The highest values of Cr and Ni were due to their presence in the parent materials amphibolite or serpentine.

PICT determination

To estimate the bacterial community tolerance to heavy metals (Cr, Ni, Pb, and Zn), 7.5 g of sieved soil were reactivated by rewetting until reaching 50–60% of water holding capacity and then incubated at 22 °C for 30 days (Meisner et al. 2013).

Bacterial community tolerance to Cr, Ni, Pb, and Zn was determined by the same procedure described for Cu in Campillo-Cora et al. (2021). To obtain bacterial suspensions, each soil sample was subdivided into three centrifuge tubes of 50 mL (2.5-g rewetted soil in each tube), and 30 mL of 20 mM MES buffer (pH 6) were added. A multi-vortex was used to mix the suspensions for 3 min. After that, a low-speed centrifugation step was performed (1000 × g, 10 min) to generate a bacterial suspension in the supernatant removing most of the fungal biomass (Bååth 1994; Bååth et al. 2001; Rousk and Bååth 2011). Soil supernatants were filtered through glass wool and single aliquots of 1.5 mL were transferred into 10 microcentrifugation tubes. A volume of 0.15 mL of different metal concentrations of Cr, Ni, Pb, and Zn was added to these tubes, to finally obtain nine concentrations (from 3.3×10^{-4} to 10^{-8} M) of each metal. A blank was established adding 0.15 mL of distilled water. Metal concentrations were made from the following metal salts: $K_2Cr_2O_7$, $Ni(NO_3)_2{\cdot}6H_2O$, $Pb(NO_3)_2$, and $Zn(NO_3)_2{\cdot}6H_2O$. Then, the ^{3}H-leucine incorporation method was used to estimate the bacterial community growth (Bååth et al. 2001). A volume of 0.2 µL [^{3}H]Leu (37 MBq mL^{-1} and 5.74 TBq mmol^{-1} Amersham) with non-labeled Leu (19.8 µL) was added to each tube, resulting in 300 nM Leu in the bacterial suspensions. After that, bacterial suspensions were incubated for 8 h at 22 °C. Bacterial growth was stopped with 75 µL of 100% trichloroacetic acid. A washing procedure was performed as described by Bååth et al. (2001), and radioactivity was measured by liquid scintillation counting using a Tri-Carb 2810TR (PerkinElmer, USA).

Data analysis

For each soil and heavy metal, a dose–response curve was obtained with the PICT methodology. The four lowest added metal concentrations had no effect on bacterial growth, i.e., no inhibition was observed. Hence, to obtain comparable dose–response curves, relative bacterial growth was calculated by dividing all data in a curve by the average of results from the four lowest added metal concentrations (including the blank) with no inhibition. From the dose–response curves, log IC_{50} was calculated as a tolerance index, i.e., the concentration that inhibits 50% of bacterial growth using a logistic model:

$$Y = \frac{c}{1 + e^{b(X-a)}}$$

Y is the measured level of Leu incorporation for each metal concentration, *X* is the decimal logarithm of metal concentration in the bacterial suspension, *a* is log IC_{50}, *c* the Leu incorporation without added metal, and *b* is a slope parameter indicating inhibition rate (Table S5; Supplementary Information) (Fernández-Calviño et al. 2011). To facilitate the interpretation of the results, IC_{50} was expressed as log IC_{50}. Bacterial community tolerance will be greater when log IC_{50} values are higher.

Statistical analysis

For each studied metal, an ANOVA (Table S6; Supplementary Information) analysis followed by Duncan's post hoc test was used to verify if soils from different parent materials showed significant differences regarding bacterial community tolerance to the heavy metal. Before that, residual normality and variance homogeneity through Shapiro–Wilk's and Levene's tests, respectively, were verified (Table S7; Supplementary Information).

In order to obtain equations to estimate the baseline bacterial community tolerance to the different heavy metals (Cr, Ni, Pb, and Zn), multiple regression analysis was performed using log IC_{50} values as dependent variables and soil properties as independent variables. Further, binary variables related to each parent material were also used. These variables will take a value of 1 or 0 depending on parent material. For example, in a binary variable for granite soils, only granite soil samples will have a value of 1, and the rest of the soil samples a value of 0. The backward elimination method was used to perform the analysis. After that, a series of determining factors were verified: linearity, error independency, residues homoscedasticity, residuals normality, autocorrelation, collinearity, and presence of outliers. Corrected coefficient of determination (R^2) was used to estimate the equations fit of goodness. All statistical tests were conducted using SPSS Statistics 25 software (IBM, USA).

Results

Bacterial community tolerance to Cr, Ni, Pb, and Zn in soils with geochemical baseline concentrations (GBCs) of heavy metals

Dose–response curves (inhibition curves) obtained for Cr, Ni, Pb, and Zn in the 30 studied soils are presented in Figs S2–S21 (Supplementary Information). The sigmoid shape obtained for most curves indicates that bacterial growth reached minimum values when added metal concentrations were highest, and as metal concentrations decreased, relative bacterial growth tended to 1. All dose–response curves were well fitted to the logistic model ($R^2 \geq 0.99$ for Cr, $R^2 \geq 0.91$ for Ni, $R^2 \geq 0.98$ for Pb, and $R^2 \geq 0.97$ for Zn), providing good estimations of log IC_{50} values (Table 1).

Chromium

A wide range of bacterial community tolerance to Cr, expressed as log IC_{50}, was found: -6.17 ± 0.05 to -3.67 ± 0.05 (Table 1). The ANOVA analysis detected significant differences in log IC_{50} values between parent materials ($p < 0.05$). Duncan's post hoc test after ANOVA

Table 1 Bacterial community tolerance to different heavy metals expressed as log IC_{50} for geochemical baseline concentrations in soils with different parent materials

Parent material	Soil	Cr Log $IC_{50} \pm$ error	R^2	Ni Log $IC_{50} \pm$ error	R^2	Pb Log $IC_{50} \pm$ error	R^2	Zn Log $IC_{50} \pm$ error	R^2
Granite (n = 6)	1	−5.13 ± 0.09	0.996	−3.86 ± 0.09	0.988	−4.61 ± 0.04	0.996	−4.18 ± 0.09	0.994
	2	−5.53 ± 0.05	0.995	−4.25 ± 0.05	0.991	−4.46 ± 0.07	0.987	−3.70 ± 0.10	0.994
	3	−4.82 ± 0.09	0.995	−4.48 ± 0.05	0.992	−4.41 ± 0.08	0.984	−3.54 ± 0.12	0.984
	4	−4.34 ± 0.06	0.996	−5.04 ± 0.09	0.996	−4.69 ± 0.06	0.993	−4.04 ± 0.07	0.988
	5	−4.40 ± 0.08	0.994	−5.39 ± 0.07	0.993	−4.56 ± 0.11	0.984	−3.67 ± 0.19	0.972
	6	−4.84 ± 0.06	0.998	−5.03 ± 0.11	0.990	−5.21 ± 0.10	0.988	−4.26 ± 0.08	0.991
Limestone (n = 6)	7	−4.52 ± 0.07	0.997	−3.74 ± 0.12	0.987	−5.24 ± 0.07	0.992	−3.99 ± 0.07	0.989
	8	−4.41 ± 0.08	0.991	−3.44 ± 0.21	0.979	−4.95 ± 0.07	0.993	−3.82 ± 0.07	0.987
	9	−4.34 ± 0.09	0.991	−3.56 ± 0.31	0.962	−4.69 ± 0.05	0.995	−3.76 ± 0.06	0.987
	10	−4.55 ± 0.09	0.992	−3.09 ± 0.12	0.973	−4.61 ± 0.07	0.992	−3.62 ± 0.06	0.981
	11	−4.25 ± 0.09	0.992	−3.62 ± 0.09	0.983	−5.23 ± 0.05	0.998	−4.02 ± 0.08	0.983
	12	−4.35 ± 0.04	0.999	−4.33 ± 0.08	0.991	−4.48 ± 0.11	0.975	−4.06 ± 0.06	0.988
Schist (n = 6)	13	−5.46 ± 0.12	0.990	−5.35 ± 0.07	0.991	−3.99 ± 0.01	0.999	−3.48 ± 0.03	0.997
	14	−5.59 ± 0.09	0.996	−5.23 ± 0.24	0.969	−4.64 ± 0.05	0.995	−4.40 ± 0.08	0.994
	15	−6.17 ± 0.05	0.993	−5.03 ± 0.05	0.995	−4.68 ± 0.06	0.995	−4.31 ± 0.07	0.993
	16	−5.07 ± 0.10	0.994	−4.55 ± 0.05	0.993	−3.69 ± 0.03	0.992	−4.32 ± 0.09	0.987
	17	−5.88 ± 0.04	0.997	−4.85 ± 0.04	0.996	−4.33 ± 0.08	0.986	−3.94 ± 0.04	0.994
	18	−5.48 ± 0.07	0.995	−5.77 ± 0.14	0.980	−4.31 ± 0.03	0.996	−3.96 ± 0.05	0.992
Amphibolite (n = 6)	19	−5.39 ± 0.09	0.991	−6.44 ± 0.06	0.996	−4.33 ± 0.08	0.982	−4.17 ± 0.05	0.994
	20	−6.00 ± 0.04	0.997	−4.69 ± 0.06	0.996	−4.19 ± 0.09	0.980	−3.95 ± 0.08	0.972
	21	−4.31 ± 0.11	0.986	−4.38 ± 0.14	0.983	−4.73 ± 0.13	0.987	−3.52 ± 0.08	0.985
	22	−5.07 ± 0.06	0.997	−5.35 ± 0.09	0.992	−4.72 ± 0.10	0.987	−4.38 ± 0.09	0.985
	23	−5.04 ± 0.12	0.989	−5.53 ± 0.24	0.961	−4.64 ± 0.07	0.993	−4.34 ± 0.06	0.993
	24	−5.48 ± 0.06	0.996	−5.79 ± 0.08	0.995	−4.60 ± 0.03	0.998	−4.32 ± 0.03	0.999
Serpentine (n = 6)	25	−5.57 ± 0.06	0.998	−4.63 ± 0.03	0.999	−4.45 ± 0.04	0.996	−3.80 ± 0.06	0.992
	26	−5.69 ± 0.07	0.996	−4.53 ± 0.05	0.995	−4.17 ± 0.02	0.998	−3.35 ± 0.08	0.982
	27	−5.52 ± 0.09	0.994	−3.89 ± 0.03	0.997	−4.18 ± 0.04	0.993	−3.85 ± 0.05	0.991
	28	−6.03 ± 0.08	0.997	−4.93 ± 0.03	0.998	−4.27 ± 0.02	0.999	−3.33 ± 0.03	0.997
	29	−4.81 ± 0.06	0.997	−3.67 ± 0.05	0.993	−4.53 ± 0.08	0.991	−3.82 ± 0.02	0.999
	30	−3.67 ± 0.05	0.996	−2.79 ± 0.43	0.905	−4.50 ± 0.09	0.991	−3.12 ± 0.08	0.982

R^2 represents the coefficient of determination from fitting dose–response curves (Figs. S2–S21; Supplementary Information) to the logistic model. Error denotes SE

showed the order of decreasing tolerance: limestone ≥ granite ≥ serpentine = amphibolite ≥ schist (Fig. 1a).

In order to estimate bacterial community tolerance to Cr in soils with GBCs as a function of soil properties, a general equation for all studied soils ($n = 30$) was calculated (Table 2). This equation explained 44% of the variance ($R^2 = 0.44$; $p < 0.001$) by including the clay fraction (positive relationship with log IC_{50}) and two binary variables: one for soils with limestone parent material and another for granite soils. The presence of a binary variable associated with a single parent material indicates that some soil properties or a combination of properties, not included in the equation, were influencing bacterial community tolerance to Cr. In addition to the low variance explained (44%), the general equation for Cr overestimated low and underestimated high log IC_{50} values (Fig. 2a).

In order to improve the estimation of bacterial community tolerance to Cr (log IC_{50} values) in soils with GBCs of heavy metals, five individual equations were calculated, one for each parent material (Table S8; Supplementary Information). The individual equations included variables such as clay fraction (positive relationship, like in the general equation), DOC (negative), soil pH (negative), and total Ni (positive). Any general similarity among the different equations was not found, i.e., different combinations of soil properties affecting log IC_{50} results were involved in soils with different parent materials. The individual equations generally increased the R^2 values (0.51–0.85)

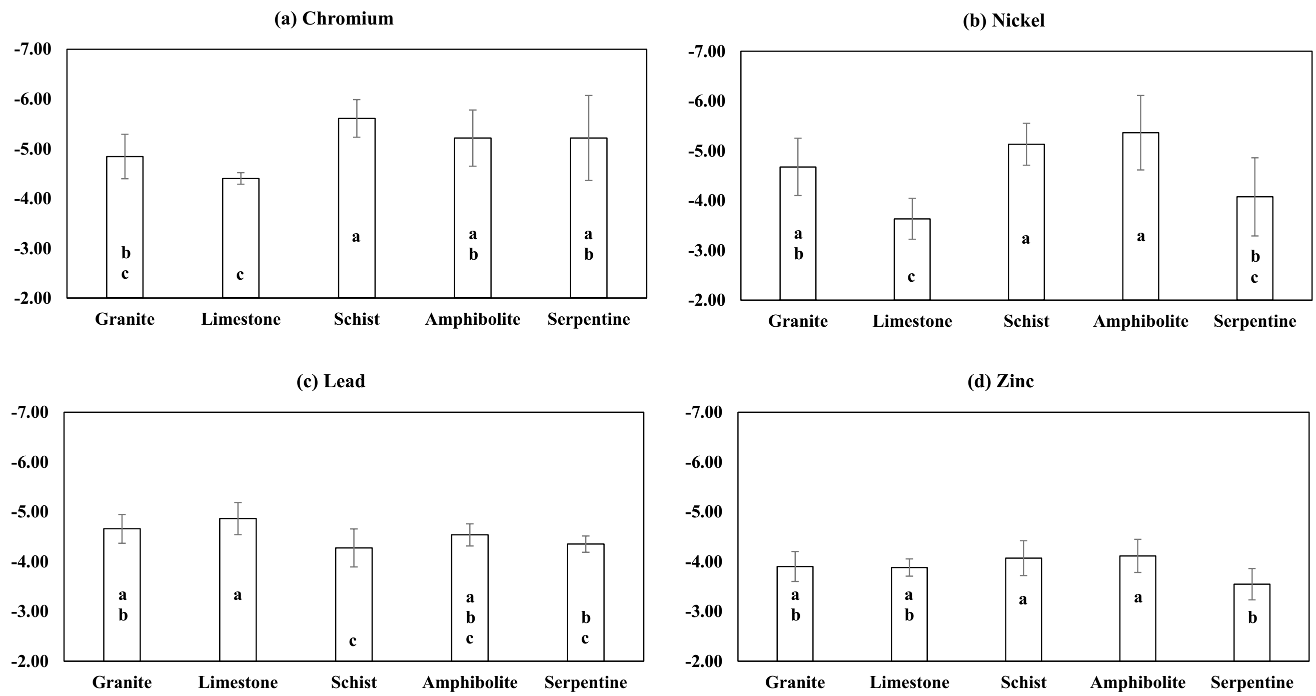

Fig. 1 Mean and standard deviation of tolerance values of the bacterial communities (log IC_{50}) to Cr (**a**), Ni (**b**), Pb (**c**), and Zn (**d**) in soils developed from granite ($n=6$), limestone ($n=6$), schist ($n=6$), amphibolite ($n=6$), and serpentine ($n=6$). ***a***, ***b***, and ***c*** indicate homogeneous subsets obtained from Duncan's test

Table 2 General equations for the estimation of bacterial community tolerance to different metal baseline levels (log IC_{50} values, $n=30$) obtained by multiple regression analysis

		R^2	F	Sig. (p-value)
Chromium	$\text{Log } IC_{50} = -(6.552 \pm 0.465) + (0.053 \pm 0.020)\textbf{Clay} + (1.141 \pm 0.238)\textbf{BV}_\textbf{L} + (0.754 \pm 0.245)\textbf{BV}_\textbf{G}$	0.44	8.66	< 0.001
	(p< 0.001) (p=0.013) (p< 0.001) (p=0.005)			
Nickel	$\text{Log } IC_{50} = -(6.112 \pm 0.491) + (0.049 \pm 0.023)\textbf{Clay} + (0.925 \pm 0.413)\textbf{DOC} - (0.048 \pm 0.020)\textbf{Al}$			
	(p< 0.001) (p=0.040) p=(0.035) (p=0.024)	0.68	12.97	< 0.001
	$+ (0.861 \pm 0.201)\textbf{BV}_\textbf{SE} + (1.162 \pm 0.275)\textbf{BV}_\textbf{L} + (0.753 \pm 0.286)\textbf{BV}_\textbf{SE}$			
	(p<0.001) (p=0.015)			
Lead	$\text{Log } IC_{50} = -(5.018 \pm 0.148) + (0.490 \pm 0.204)\textbf{DOC} + (0.094 \pm 0.028)\textbf{NiCr} + (0.451 \pm 0.013)\textbf{BV}_\textbf{S}$	0.36	4.95	0.005
	(p< 0.001) (p=0.024) (p=0.002) (p=0.002)			
	$+ (0.253 \pm 0.135)\textbf{BV}_\textbf{A}$			
	(p=0.073)			
Zinc	$\text{Log } IC_{50} = -(2.932 \pm 0.586) + (0.023 \pm 0.012)\textbf{Clay} - (0.344 \pm 0.138)\textbf{pH} + (1.165 \pm 0.405)\textbf{BV}_\textbf{L}$	0.38	5.24	0.004
	(p< 0.001) (p=0.075) (p=0.020) (p=0.008)			
	(p< *0.001)*			

Clay, clay fraction; *DOC*, dissolved organic carbon; *Al*, free Al; *NiCr*, sum of Ni and Cr total content; *pH*, soil pH measured in water; $BV_{(L, G, SE, S, A)}$, binary variables for limestone (L), granite (G), serpentine (SE), schist (S), and amphibolite (A). Values in brackets below parameter values are p-values for each variable included in the models. R^2 represents corrected coefficient of determination. Error denotes SE

compared to the general Eq. (0.44); but for limestone parent material an individual equation was not possible to achieve, probably due to the low variation in log IC_{50} values (0.30 units between maximum and minimum).

The relationship between measured and estimated log IC_{50} values using both the general equation and the individual equations were compared (Fig. 2). It should be noted that for the limestone soils, we used the mean value of the six

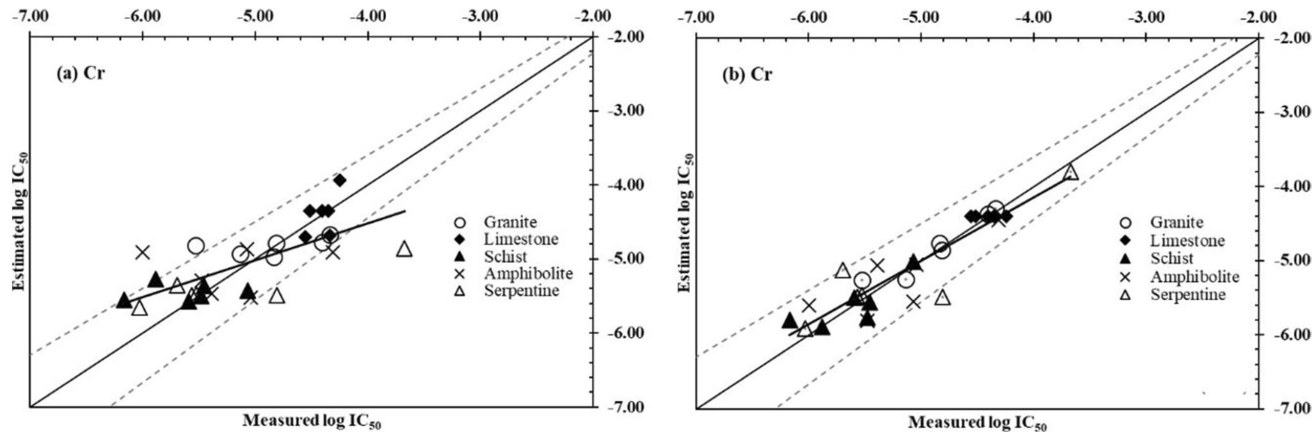

Fig. 2 Measured tolerance values of bacterial communities (log IC_{50}) for Cr versus estimated tolerance values determined from **a** the general equation and **b** individual equations for each soil parent type. The continuous line represents a 1:1 relationship, whereas stippled lines represent 10% deviation from the 1:1 line

samples (−4.40) as a constant number for tolerance. Individual equations predicted log IC_{50} values better, over- and underestimations were lower (but with the same tendency) than with the general equation and all log IC_{50} values estimated using individual equations were within 10% of the measured log IC_{50} values.

Nickel

The range for log IC_{50} values (bacterial community tolerance) was between -6.44 ± 0.09 and -2.79 ± 0.43 for Ni, the largest range among the studied metals (Table 1). The ANOVA analysis showed significant differences in bacterial community tolerance to Ni between different parent material groups ($p < 0.05$), while Duncan's post hoc test showed the following order of decreasing tolerance: limestone > serpentine ≥ granite ≥ amphibolite = schist (Fig. 1b).

The general Ni log IC_{50} equation was calculated as a function of clay fraction (positive relationship), DOC (positive), free Al (negative), and two binary variables related to limestone and serpentine (Table 2; $R^2 = 0.68$; $p < 0.001$). Despite the high variance explained (68%), the general equation overestimated low and underestimated high log IC_{50} values, and many values presented a higher error than 10% (Fig. 3a).

Individual equations for log IC_{50} estimations, one for each parent material, increased R^2 values for all parent materials (0.70–0.997) compared to the general Eq. (0.68) (Table S9; Supplementary Information). Variables included in individual equations were soil pH (negative relationship), DOC (positive), OM (negative), clay content (positive), free Fe (negative), or total Ni (positive). The equations for different parent materials presented in most cases common variables, such as clay (four equations) or DOC, pH, and OM

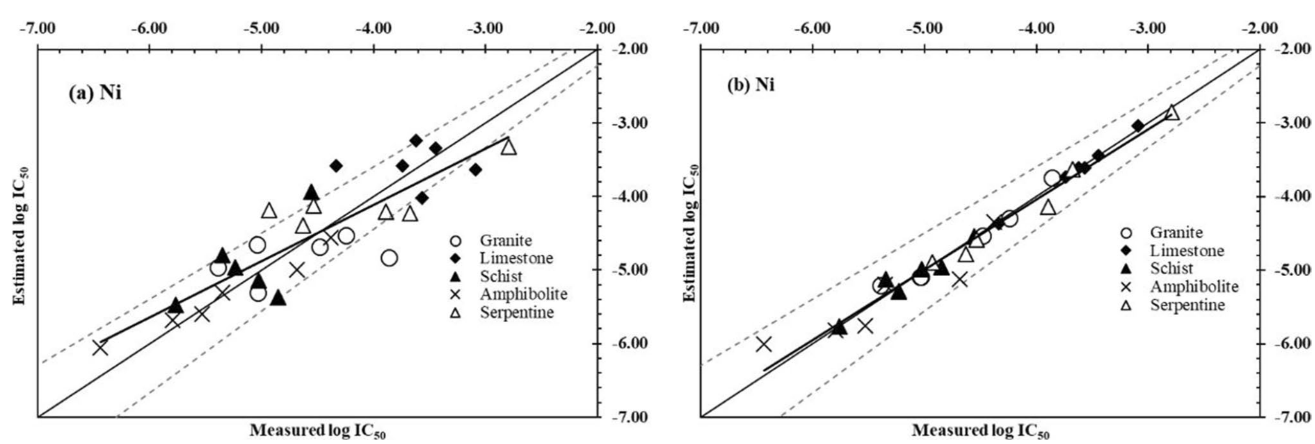

Fig. 3 Measured tolerance values of bacterial communities (log IC_{50}) for Ni versus estimated tolerance values determined from **a** the general equation and **b** individual equations for each soil parent type. The continuous line represents a 1:1 relationship, whereas stippled lines represent 10% deviation from the 1:1 line

(three equations each). When comparing the results obtained with the general (Fig. 3a) and individual equations (Fig. 3b), individual equations were better adjusted to a 1:1 line (measured vs. estimated), i.e., a more accurate estimate than the general equation. In addition, no over- nor underestimations were present, and all values presented errors lower than 10% (Fig. 3b).

Lead

Bacterial community tolerance to Pb, expressed as log IC_{50}, showed a smaller range than for Ni and Cr: -5.24 ± 0.07 to -3.69 ± 0.03 (Table 1). The ANOVA analysis and Duncan's post hoc test showed significant differences ($p < 0.05$) between parent materials, following the order: schist ≥ serpentine ≥ amphibolite ≥ granite ≥ limestone (Fig. 1c).

The log IC_{50} values could be estimated ($R^2 = 0.36$; $p < 0.05$) from DOC (positive relationship), NiCr (sum of total Ni and Cr amounts in $g \cdot kg^{-1}$) (positive), and two binary variables associated with schist and amphibolite soils (BV_S, BV_A) (Table 2). Despite the relatively low variance explained (36%), the general equation for Pb presented all values with errors lower than 10%. However, the equation overestimated low and underestimated high log IC_{50} values.

Individual equations with higher R^2 values than the general equation ($R^2 = 0.36$) were obtained for granite ($R^2 = 0.62$) and schist soils ($R^2 = 0.73$), but it was not possible to estimate any equation for limestone, amphibolite, and serpentine soils (Table S10; Supplementary Information). The variables included in the two estimated equations were DOC (positive relationship) for granite, and clay fraction (positive), and soil pH (negative) for schist. Any general similarity among the two estimated equations was not found. Amphibolite and serpentine soils showed little variation in log IC_{50} values (0.5 and 0.4, respectively), but in the case of limestone soils, log IC_{50} values had a higher variation (0.8). Even without 3 individual equations, and thus using the mean log IC_{50} value for these metals, it appeared that individual equations were better predictors for log IC_{50} values than the general equation (Fig. 4). Estimated log IC_{50} values from individual equations (Fig. 4b) fitted better to the line 1:1, i.e., presented lower overestimations at low and underestimations at high log IC_{50} values than the general equation (Fig. 4a).

Zinc

The smallest variation in log IC_{50} values was observed for Zn: -4.40 ± 0.08 to -3.12 ± 0.08 (Table 1). The ANOVA analysis showed significant differences between parent materials ($p < 0.05$) with the following order of tolerance according to Duncan's post hoc test: serpentine ≥ limestone = granite ≥ amphibolite = schist (Fig. 1d).

A general equation was obtained to estimate bacterial community tolerance to Zn as log IC_{50} values (Table 2) using clay content (positive relationship), soil pH (negative), and two binary variables associated with limestone (BV_L) and serpentine soils (BV_{SE}) ($R^2 = 0.38$). In addition to the low variance explained (38%), the general equation for Zn overestimate low and underestimate high log IC_{50} values (Fig. 2a), and some estimates had errors higher than 10%.

Individual equations for each parent material (Table S11; Supplementary Information) showed higher R^2 values than the general equation in the case of limestone ($R^2 = 0.99$), amphibolite ($R^2 = 0.90$) and serpentine soils ($R^2 = 0.80$). These equations included variables such as organic matter content (negative relationship), soil pH (negative), DOC (positive), and free Fe (negative). In three of the fitted equations DOC is present, while pH is present in two of them. It was not possible to obtain individual equations for granite

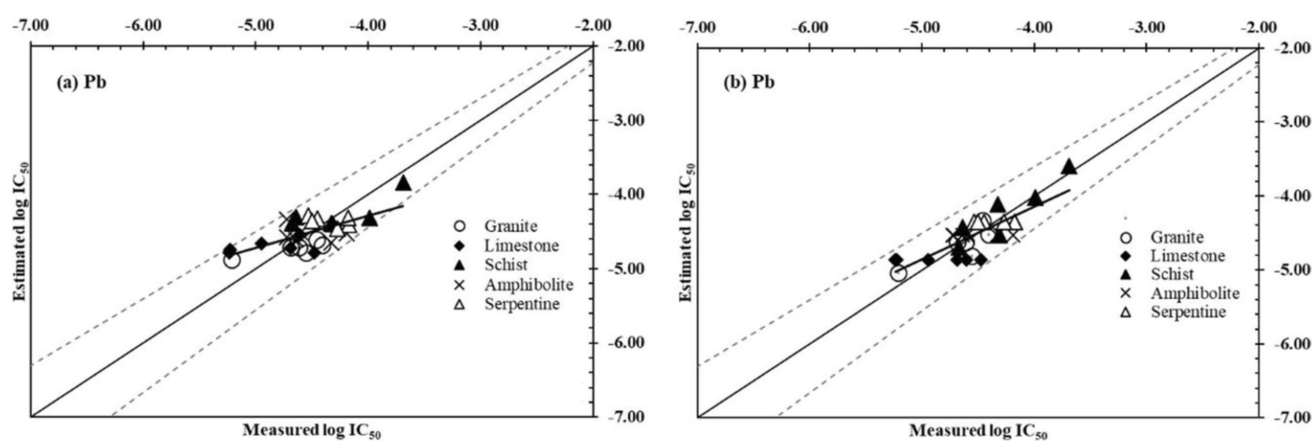

Fig. 4 Measured tolerance values of bacterial communities (log IC_{50}) for Pb versus estimated tolerance values determined from **a** the general equation and **b** individual equations for each soil parent type. The continuous line represents a 1:1 relationship, whereas stippled lines represent 10% deviation from the 1:1 line

and schist soils despite the wide range of log IC_{50} values (0.72 and 0.92, respectively). Using the mean log IC_{50} values for granite and schist, the predictions provided by individual equations (Fig. 5b) were similar to the general equation (Fig. 5a).

Discussion

In soils with GBCs of heavy metals, i.e., not polluted, the same, low, bacterial community tolerance to heavy metals is expected. However, the present work showed that differences may be present in bacterial community tolerance to Cr, Ni, Pb, and Zn in soils with different soil properties despite GBCs, as was previously shown for Cu (Campillo-Cora et al. 2021). Community tolerance to Zn and Pb had a similar variation between soils as earlier found for Cu, while Cr and Ni had a much higher variation. This difference may theoretically be attributed to high natural levels of Cr and Ni in amphibolite or serpentine soils (Chrysochoou et al. 2016; Massoura et al. 2006; Oze et al. 2004; Vithanage et al. 2014). However, amphibolite and serpentine soils did not show especially high community tolerance to Cr or Ni compared to the other soils. Instead, higher tolerance was found for limestone soils, soils with lower total (Campillo-Cora et al. 2021), and much lower available (Table S4; Supplementary Information) Cr and Ni concentrations. These results indicated that bacterial community tolerance to these metals did not mainly depend on the metal content, but that other soil properties influenced the measurements. In the cases of Pb and Zn, all soils had low total and available (Table S4; Supplementary Information) concentrations and differences in log IC_{50} values had to be due to variations in other soil properties.

Effect of soil properties on estimated bacterial community tolerance to Cr, Ni, Pb, and Zn

The effect of the general soil properties on bacterial community tolerance measurements can be divided into two groups: (1) soil properties affecting bacterial community tolerance development during the selection phase (total Ni, total NiCr, soil pH, organic matter, free Al, and free Fe contents) and (2) soil properties affecting bacterial community tolerance measurement during the detection phase (clay and DOC).

For all metals and parent soils, clay content was positively related to log IC_{50} values, suggesting that the effect was similar for all heavy metals tested (Table 2). To exemplify with Cr, this positive relationship is not possible during the selection phase. Clay minerals have a great affinity for Cr (Loyaux-Lawniczak et al. 2001), and therefore, higher amounts of clay should decrease Cr toxicity in soil, leading to lower log IC_{50} values. However, during the detection phase of PICT, the presence of high amounts of clay may lead to a detection bias. When bacteria are extracted from soil, the finer clay fractions may also be extracted. Metals and clay minerals will bind, leading to less bioavailability for bacteria. This will cause an underestimation of the Cr toxicity to bacterial communities in the detection phase, and therefore to overestimations of bacterial community tolerance. This type of artifact was previously suggested for bacterial community tolerance to Cu (Campillo-Cora et al. 2021; Fernández-Calviño et al. 2011). A bias in the detection phase of PICT is probably also the case for the other metals (Table 2) because of the high affinity of clay for heavy metals, e.g., Ni (Zhang et al. 2015) and Zn (Shaheen et al. 2013).

A positive relationship was determined between DOC and Ni, Pb and Zn log IC_{50} values (Table 2). Due to the high affinity of DOC for heavy metals, e.g., for Ni (Lockwood et al. 2015) and Pb (Bérard et al. 2016; Boivin et al. 2006;

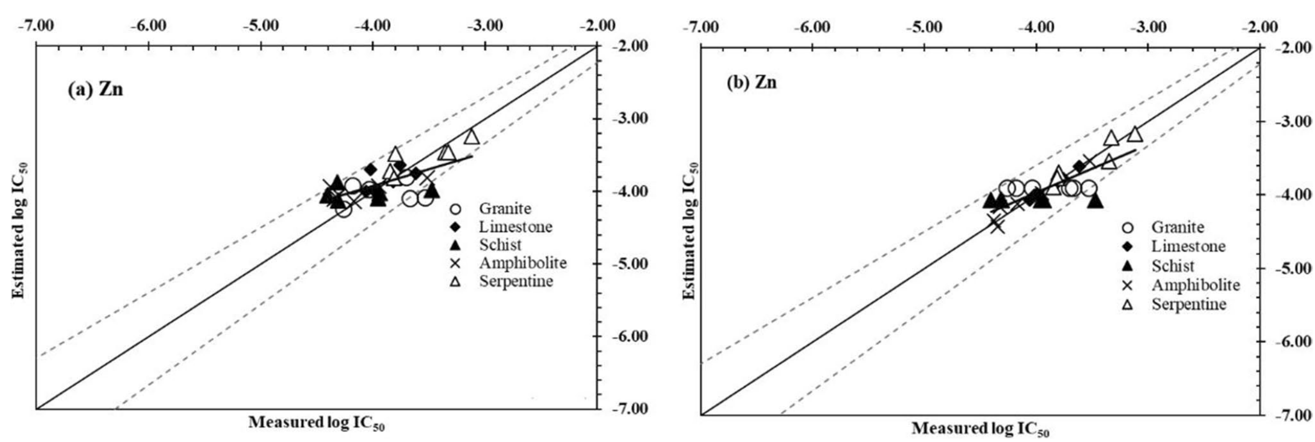

Fig. 5 Measured tolerance values of bacterial communities (log IC_{50}) for Zn versus estimated tolerance values determined from **a** the general equation and **b** individual equations for each soil parent type. The continuous line represents a 1:1 relationship, whereas stippled lines represent 10% deviation from the 1:1 line

Yamada and Katoh 2020), positive relations are not possible during the selection phase, similar to the case with the clay fraction. However, similar to clay, DOC will also be extracted into the bacterial solution in the detection phase, reducing considerably metal bioavailability and toxicity. As a result, PICT values may be overestimated, as previously found for Cu (Campillo-Cora et al. 2021; Lekfeldt et al. 2014). However, for Cr, DOC was negatively related to log IC_{50}, suggesting that DOC increase Cr toxicity for bacteria during the detection phase. This may be due to the presence of Cr in anionic form ($Cr_2O_7^{2-}$), while the other tested metals were present in cationic form (M^{2+}). However, further analyses are needed to clarify the effect of DOC on Cr toxicity during the detection phase.

A positive relationship was found between the sum of total amounts of Ni and Cr and Pb (log IC_{50}). Positive relationships were also found between Cr log IC_{50} and total Ni and between Ni log IC_{50} and total Ni. These relations may be explained by bacterial interactions with Cr and/or Ni during the selection phase of PICT. When Cr and Ni are present in soils in high concentrations, as in the case of serpentine soils, they may become toxic to soil bacteria, increasing bacterial community tolerance to Cr and Ni but also to other heavy metals. Cr and Ni toxicity may also increase bacterial community tolerance to other metals due to the co-tolerance phenomenon (Blanck 2002; Boivin et al. 2002). Previously, Díaz-Raviña et al. (1994) found co-tolerance between metals. Their results did, however, not show that Pb developed co-tolerance to Ni, Cd, Zn, or Cu. Rusk et al. (2004), on the other hand, determined that prior exposure of soil microorganisms to Zn resulted in greater tolerance to Pb and vice versa.

Free Al showed a negative relationship with estimated Ni log IC_{50} values, i.e., the higher the free Al content, the lower the bacterial community tolerance to Ni. This may be explained by the positive relation between Al oxy-hydroxide content and its Ni adsorption capacity (Zhang et al. 2015). If Ni is present in soils with high free Al concentrations, Ni becomes less bioavailable than in soils with low free Al content, leading to less toxicity on bacteria and therefore less bacterial community tolerance to Ni. In some of the individual equations, a negative relationship was found between Ni log IC_{50} values and organic matter (OM) and/or free Fe contents. The interpretation for these relationships may be the same as for free Al, i.e., an effect during the selection phase since OM and Fe oxy-hydroxides both have a high capacity for heavy metal adsorption (Bradl 2004).

A negative relationship was observed between soil pH and Zn log IC_{50} in the general equation. This relationship may be attributed to a pH effect on metals during the selection phase of PICT. There is a close relationship between soil pH and Zn bioavailability (Campillo-Cora et al. 2020; Sauvé et al. 2000), in that the higher the soil pH, the lower is the Zn bioavailability and toxicity. Therefore, bacterial community tolerance will be lower than expected from total Zn concentrations at high pH. However, Zn concentrations in the studied soils are quite low (Campillo-Cora et al. 2021) and bacterial community tolerance to Zn should thus be low independently of soil pH. Nevertheless, soil pH also has an important effect on the availability of other metals, such as Ni (Campillo-Cora et al. 2020), and therefore co-tolerance mechanisms may be present as were discussed previously for Pb (see above).

Applicability and limitations

In a previous work (Campillo-Cora et al. 2021), we suggested a methodology, based on baseline predictions of Cu tolerance of the bacterial community using soil properties, to decide if Cu pollution has a toxic effect on the bacterial community. The procedure was as follows: (1) determination of the bacterial community tolerance to Cu in a soil; (2) estimation of the bacterial community tolerance to Cu baseline using the general soil properties; and (3) calculation of the difference between estimated baseline and measured log IC_{50} in the polluted area. The resulting difference is PICT.

Results from the present work indicated that the proposed methodology may also be applied to other heavy metals. Preferentially the community tolerance should have low variation between soils with GBCs or a large part of the variation explained by few soil properties. This was the case with Cu earlier studied, with a variation of 1.6 units and 80% of the variation explained by soil properties (Campillo-Cora et al. 2021). However, even if the variation in log IC_{50} values was similar for Zn and Pb (1.3 and 1.6 units, respectively) compared to Cu, the lower variation explained (36–38%) made the baseline estimate for Zn and Pb less precise. This was even more evident for Cr with a variation of 2.5 units but only 44% of that variation was explained. The highest variation in community tolerance explained by the soil properties (68%) was obtained for Ni, but due to also having the highest variation in tolerance values, a similar high absolute variation in tolerance values as for Cr was found. Thus, although none of the metals studied showed as good results for baseline estimated tolerance as earlier found for Cu, the methodology should be applicable also for Zn and Pb, but improvements are necessary in the case of Ni and Cr.

High variation and low explanation due to soil properties could be due to two different problems. The actual measurements could differ between metals, resulting in less precise IC_{50} determinations. However, there was no obvious difference in determination precision with high R^2 values for the dose–response curves for all metals. Thus, it appears that compared to Cu, there are other soil properties that are important for the determination of baseline tolerance values, especially for Ni and Cr; properties that need to be identified before the proposed methodology based on baseline predictions can be applied for these metals. One such property could be clay composition since clays are a wide group of minerals with highly variable properties (Brady and Weil 2002). Other not studied parameters such as the presence of carbonates or phosphates may also be relevant.

Another problem was that all general equations overestimated low and underestimated high log IC_{50} values. However, independent equations for different parent materials may improve the variance explained and reduce log IC_{50} values over- and underestimations. However, the low number of soils ($n = 6$) used for the log IC_{50} baseline estimation for each parent material so far leads to low robustness.

The regression models obtained in the present work are limited to the leucine incorporation method during the PICT detection phase. Although sensitive and easy to use, the method is not applicable to all laboratories due to the need for authorization to work with radioactive isotopes. Therefore, further research, based on the same methodology proposed in the present work but using other endpoints during the detection phase, is also needed. Previously, enzymatic activities (Aliasgharzad et al. 2011), methods based on respiration (Bérard et al. 2016; Wakelin et al. 2014) or on community-level physiological profiling (CLPP) (Schmitt et al. 2004) were for example used to determine PICT to contaminants in soils.

Despite the limitations discussed above, the general methodology proposed by Campillo-Cora et al. (2021) for Cu and extended to Cr, Ni, Pb, and Zn in the current work, open new possibilities to improve the detection of toxic effects caused by heavy metals in soils from polluted areas. However, before routine application of the proposed methodology for the estimation of baseline levels of bacterial community tolerance to heavy metal, the study of individual equations for different parent materials using more soil samples is needed to achieve more robust equations, especially for Ni and Cr. These samples must include a higher variability of soil properties and more parent materials. In addition, the obtained equations must be validated with a new independent set of samples.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-021-01604-x.

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Declarations

Competing interests The authors declare no competing interests.

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