Geoderma 430 (2023) 116335

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Dissolved organic matter as a confounding factor in the determination of pollution-induced community tolerance (PICT) of bacterial communities to heavy metals using the leucine incorporation method

Claudia Campillo-Cora *, Rocío González-Feijóo, Manuel Arias-Estévez, David Fernández-Calviño

Departamento de Bioloxía Vexetal e Ciencia do Solo, Facultade de Ciencias, Universidade de Vigo, As Lagoas s/n, 32004 Ourense, Spain

ARTICLE INFO

Keywords:
Bacterial growth
Heavy metal
3H leucine incorporation
PICT (Pollution-Induced Community Tolerance)
Dissolved organic matter (DOM)

ABSTRACT

PICT methodology using the leucine incorporation method (Leu-PICT) is useful for assessing heavy metal contamination in soils. First, bacterial community is exposed to metal in the soil (selection phase), developing tolerance if metal exerts toxicity. Secondly, in detection phase, bacterial suspensions are obtained, and tolerance is quantified by a second exposition of bacterial community to the metal using Leu-PICT methodology. However, during detection phase when Leu-PICT is performed, some characteristics of bacterial suspensions may change metal bioavailability. We assess the influence of dissolved organic matter (DOM) in bacterial suspensions, as humic acids (HA), on Leu-PICT determination to Cr, Cu, Ni, Pb and Zn. Results showed that the presence of HA in bacterial suspensions causes underestimations of bacterial community tolerance to Cr (increasing Cr toxicity), and overestimations of bacterial community tolerance to Cu, Ni, Pb, and Zn (reducing metal toxicity). In addition, the magnitude of these overestimations was different depending on the metal.

The pollution-induced community tolerance (PICT) is a sensitive technique and a promising tool that allows to infer chemical-induced structural impacts and identify the pollutant that affect microbial community structure (Brandt et al., 2010; Tlili et al., 2016). PICT is based on the development of microbial community tolerance in response to metal toxicity by means of the selection and adaptation of the species which conform the community: the most sensitive disappear and the tolerant ones dominate after exposure to a toxic substance, leading to a more tolerant community (Blanck, 2002). PICT methodology is constituted by two phases: a) selection, where the microbial community is exposed to a toxic substance; and b) detection, where microbial community tolerance is quantified through a second exposition to the toxicant by a short time-assay (Blanck, 2002). Different endpoints can be used during the detection phase, such as methods based on respiration (Bérard et al., 2016; Batista et al., 2020), enzymatic activities (Aliasgharzad et al., 2011) or community-level physiological profiling (CLPP) (Schmitt et al., 2004). One well-established, highly sensitive and economical method that allows a large number of samples to be processed is the bacterial growth using the ^{3}H-leucine incorporation (Leu-PICT) (Brandt et al., 2009; Imfeld et al., 2011). The Leu-PICT methodology was previously applied to assess several pollutants, for example, heavy metals (Brandt et al., 2010; Fernández-Calviño and Bååth, 2016), and consists of adding a range of metal concentrations to a bacterial suspension extracted from soil previously exposed to metal. Then, ^{3}H-leucine is added to the bacterial suspensions and, after an incubation period (hours), the rate of incorporation of ^{3}H-leucine is measured (Bååth et al. 2001). Despite PICT advantages, some bias during the PICT detection phase may lead to over- or underestimate tolerance measurements, e.g. such as different pH values or the presence of dissolved organic matter (DOM) as it was suggested for Cu (Lekfeldt et al., 2014). That is, if some characteristic of bacterial suspension reduces the availability of added metal, i.e. metal toxicity, then bacterial communities became exposed to a lower amount of metal, so that the determined tolerance will be higher (overestimated). The opposite occurs if some characteristic of the bacterial suspension increases the toxicity of the added metal (underestimated tolerance). In the case of DOM, when metal concentrations are added to bacterial suspensions, DOM may complex with metals, reducing their bioavailability for bacteria, resulting in overestimated measurements of bacterial community tolerance (Lekfeldt et al., 2014). Previously, Fernández-Calviño and Bååth (2016) and Lekfeldt et al. (2014)

---

* Corresponding author.
E-mail address: ccampillo@uvigo.es (C. Campillo-Cora).

https://doi.org/10.1016/j.geoderma.2023.116335
Received 6 April 2022; Received in revised form 5 January 2023; Accepted 12 January 2023

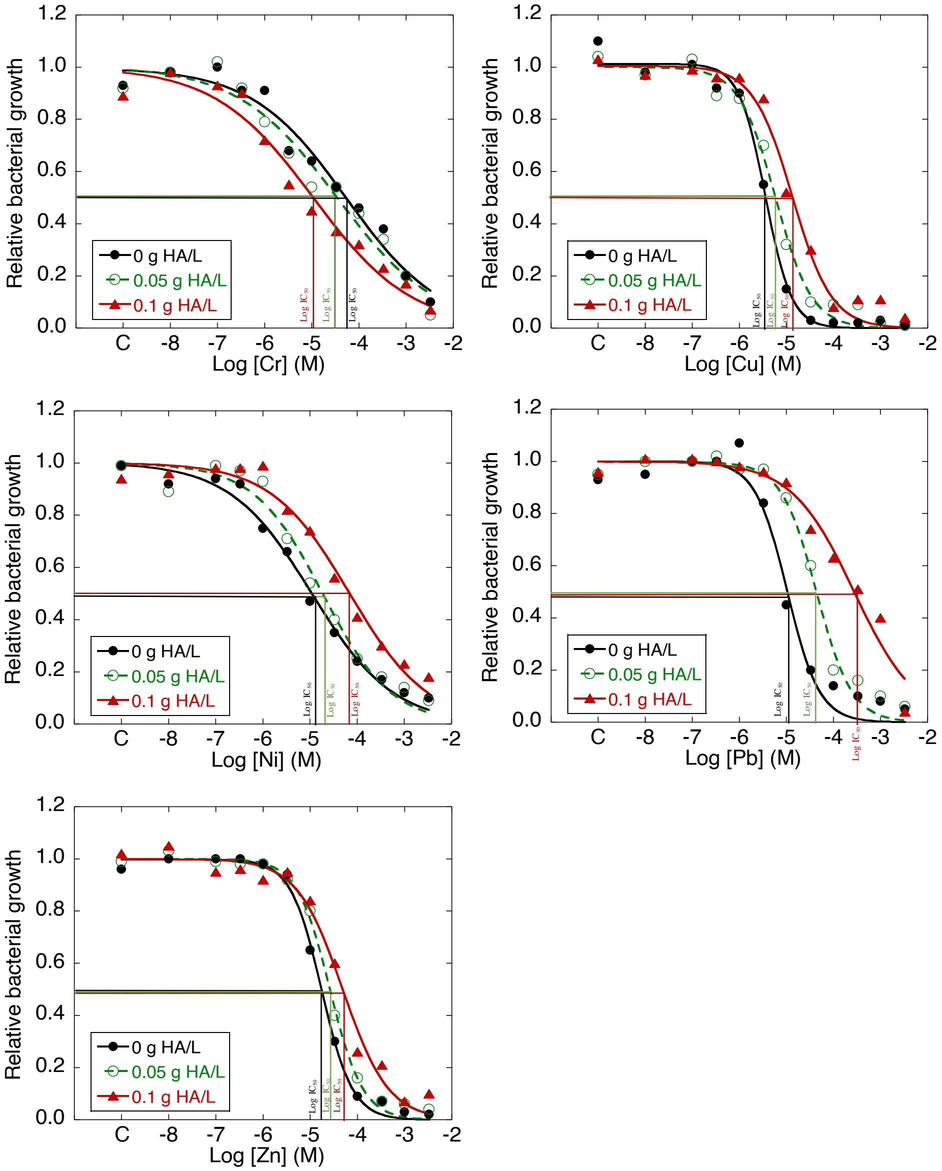

Fig. 1. Inhibition curves to Cr, Cu, Ni, Pb and Zn: effect of humic acids addition (0.1, 0.05 and 0 g/L) in bacterial growth inhibition curves.

suggested this DOM-artifact on bacterial community tolerance to Cu detection using Leu-PICT. DOM-artifact was also suggested for microbial community tolerance to Pb detection using MicroResp™ (Bérard et al., 2016). In a different study, Pradhan et al. (2016) found that Cu oxide nanoparticles exerted less microbial toxicity in presence of humic acids, suggesting that DOM reduces Cu availability. However, the effects of the presence of DOM in bacterial suspensions on the determination of PICT have not yet been demonstrated nor quantified for different metals. We hypothesized that DOM presence in bacterial suspensions leads to overestimated bacterial community tolerance measurements to heavy metals (higher apparent tolerance values). We also hypothesized that the DOM effect in bacterial community tolerance is proportional to DOM concentration. We aim to assess the magnitude of DOM-artifact on Leu-PICT detection to Cr, Cu, Ni, Pb, and Zn as a function of studied metal and DOM concentration.

Soil sampling was performed in a forest area in NW Spain. Once in the laboratory, the soil sample was air-dried, sieved (2 mm mesh size) and stored until analysis. The soil sample (pH 7.8; 0.003 g·L^{-1} DOM in bacterial suspensions) was rewetted and incubated for one month to ensure microbial recovery (Meisner et al., 2013). After the incubation period, bacterial suspensions were obtained (Bååth et al., 2001; Lekfeldt et al., 2014) and transferred (1.35 mL) to 2 mL micro-tubes (see Supplementary Material). Two humic acids (HA) concentrations were added to these micro-tubes (0.075 mL), obtaining final added soluble concentrations of 0.05 or 0.1 g HA·L^{-1}, besides a blank from distilled water (0 g HA·L^{-1}). For each HA concentration, a volume of different Cr, Cu, Ni, Pb, and Zn solutions (0.075 mL) was added to obtain eleven metal concentrations (10^{-8} to 3·10^{-3} M), plus a blank of distilled water. Each metal was added individually and in triplicate to micro-tubes. Bacterial growth was estimated in bacterial suspensions, i.e., in each micro-tube, by the ^{3}H-leucine incorporation technique. Bacterial growth data resulted in dose–response curves for each metal and HA concentration, and log IC$_{50}$ values were estimated from these curves (as a tolerance index). High log IC$_{50}$ values mean higher tolerance and vice versa. To compare the effects of HA concentrations on PICT, logIC$_{50}$ confidence intervals overlapping was analyzed.

In the inhibition curves it can be appreciated that the HA effect is different depending on the considered metal (Fig. 1). Contrary to our hypothesis, the bacterial community presented less apparent tolerance to Cr as HA concentration increases (bacterial community tolerance to Cr underestimation), i.e. bacterial communities seem more sensitive to Cr as the HA increasing in the bacterial suspension. All Cr inhibition

Table 1
Bacterial community tolerance (expressed as Log IC_{50} ± standar error) values for each studied metal (Cr, Cu, Ni, Pb and Zn) and humic acid concentration (0.10, 0.05 and 0 g/L). Different letters show significant differences according confidence intervals.

	Humic acid concentration (g/L)					
	0		0.05		0.10	
Metal	Log IC_{50} ± error	R^2	Log IC_{50} ± error	R^2	Log IC_{50} ± error	R^2
Cr	−4.27 ± 0.15^a	0.99	−4.47 ± 0.20^a	0.98	−4.97 ± 0.19^b	0.99
Cu	−5.61 ± 0.03^a	0.99	−5.23 ± 0.06^b	0.99	−4.87 ± 0.07^c	0.99
Ni	−4.96 ± 0.10^a	0.99	−4.73 ± 0.12^b	0.99	−4.16 ± 0.11^c	0.99
Pb	−4.98 ± 0.08^a	0.99	−4.36 ± 0.06^b	0.99	−3.56 ± 0.10^c	0.98
Zn	−4.76 ± 0.03^a	0.99	−4.58 ± 0.03^b	0.99	−4.31 ± 0.08^c	0.99

curves were well fitted to the logistic model ($R^2 \geq 0.98$) (Table 1). Log IC_{50} values decreased with HA concentrations, achieving a decrease of 0.2 (not significant) and 0.70 (significant) log units for 0.05 and 0.1 g HA L^{-1}, respectively (Table 1). When HA concentration increases, bacterial community tolerance apparently decreases i.e. Cr exerted more toxicity on bacterial communities in presence of HA. In this study, $K_2Cr_2O_7$ concentrations were added to bacterial suspensions, i.e. as Cr^{6+}. Cr^{+6} is more harmful to bacteria and other organisms than Cr^{+3}, and Cr^{+6} presents a great interaction capacity with dissolved organic matter, reducing its toxicity by reduction processes or forming various organic complexes (Laborda et al., 2007). However, our results were not as expected, since Cr was apparently more toxic for bacteria as HA levels increased (Table 1, Fig. 1). Cr was added as $K_2Cr_2O_7$ to bacterial suspensions and, as HA concentration increases the percentage of $Cr_2O_7^{-2}$ in bacterial suspensions slightly increases (Table S1, Supplementary Information), increasing Cr toxicity. A possible explanation is that the presence of humic acids enhances the reduction of Cr^{+6} to Cr^{+3} (Wittbrodt and Palmer, 1997), but also free radicals are formed during this process (Kotaś and Stasicka, 2000), and increasing the general toxicity for bacteria in the suspension. Another possible explanation may be the capacity of formed Cr^{+3} to coordinate various organic compounds, resulting in inhibition of some metalloenzyme systems (Kotaś and Stasicka, 2000). According to our hypothesis, inhibition curves for Cu, Ni, Pb, and Zn showed an apparent bacterial community tolerance increase as HA concentration increases, i.e. bacterial communities seems more tolerant to Cu, Ni, Pb and Zn in presence of HA in bacterial suspension (Fig. 1), overestimating PICT measurements. When metal concentrations were added to bacterial suspensions in the Leu-PICT detection phase, metal may bind to dissolved organic matter (DOM) forming metal-organo complexes, hence reducing its bioavailability (Hernandez-Soriano and Jimenez-Lopez, 2012). It is due to the affinity between heavy metals and DOM (Borggaard et al., 2019). As DOM increases, free cationic metals availability decreases (Table S1, Supplementary Information). Thus, a false bacterial community tolerance increase will be measured, i.e. tolerance measurements will be overestimated. All curves were well fitted to the logistic model: $R^2 = 0.99$ for Cu, Ni and Zn; and $R^2 \geq 0.98$ for Pb (Table 1). The addition of 0.05 g HA L^{-1} to the bacterial suspension caused a significant increase of log IC_{50} in 0.38, 0.23, 0.62 and 0.18 log units for Cu, Ni, Pb and Zn, while the addition of 0.10 g HA L^{-1} caused a significant increase of log IC_{50} in 0.74, 0.80, 1.42 and 0.45 log units for Cu, Ni, Pb and Zn. Thus the effect of HA on the bacterial community PICT follows the next sequence Pb > Cu ≈ Ni > Zn. These differences between metals may be attributed to the different metal affinities for DOM. Liu et al. (2007) reported that Pb showed the highest affinity for DOM, followed by Cu and Ni, and Refaey et al. (2014) reported that Cu is stronger adsorbed with DOM than Zn. Our study confirms, as suggested in previous works (Lekfeldt et al., 2014; Bérard et al., 2016; Campillo-Cora et al., 2021; 2022), that the presence of dissolved organic matter in bacterial suspensions causes deviations in bacterial community tolerance to heavy metals determination using ^3H leucine incorporation method as bacterial growth endpoint. In addition, results showed that the magnitude and type of deviation (over- or underestimation) depended on the studied heavy metal. As main conclusion, the present study confirms the existence of an artifact (DOM presence in bacterial suspensions) that affects the measurement of bacterial community tolerance to 5 heavy metals, which can lead to misinterpretations of tolerance measurements. In addition, for first time the effect of DOM was quantified for five heavy metals showing important differences depending on the studied metal. This raise new scientific questions and call for additional basic research, and it is of relevance for future (Leu-)PICT studies in order to correctly interpreting bacterial community tolerance results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This study has been funded by the Spanish Ministry of Economy and Competitiveness through the project CTM2015-73422-JIN (FEDER Funds). David Fernández Calviño holds a Ramón y Cajal contract (RYC-2016-20411) financed by the Spanish Ministry of Economy, Industry and Competitiveness. Claudia Campillo-Cora holds a predoctoral fellowship with Xunta de Galicia (ED401A-2020/084) funded by Consellería de Educación, Universidade e Formación Profesional. Funding for open access charge: Universidade de Vigo/CISUG.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116335.

References

Aliasgharzad, N., Molaei, A., Oustan, S., 2011. Pollution induced community tolerance (PICT) of microorganisms in soil incubated with different levels of Pb. Int. J. Environ. Ecol. Eng. 5, 838–842.

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biol. Biochem. 33, 1571–1574.

Batista, D., Tlili, A., Gessner, M.O., Pascoal, C., Cássio, F., 2020. Nanosilver impacts on aquatic microbial decomposers and litter decomposition assessd as pollution-induced community tolerance (PICT). Environ. Sci. Nano 7, 2130–2139.

Bérard, A., Capowiez, L., Mombo, S., Schreck, E., Dumat, C., Deola, F., Capowiez, Y., 2016. Soil microbial respiration and PICT responses to an industrial and historic lead pollution: a field study. Environ. Sci. Pollut. Res. 23, 4271–4281.

Blanck, H., 2002. A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. Hum. Ecol. Risk Assess. 8, 1003–1034.

Borggaard, O.K., Holm, P.E., Strobel, B.W., 2019. Potential of dissolved organic matter (DOM) to extract As, Cd Co, Cr, Cu, Ni, Pb and Zn from polluted soils: a review. Geoderma 343, 235–246.

Brandt, K.K., Sjøholm, O.R., Krogh, K.A., Halling- Sørensen, B., Nybroe, O., 2009. Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. Environ. Sci. Technol. 43, 2963–2968.

Brandt, K.K., Frandsen, R.J.N., Holm, P.E., Nybroe, O., 2010. Development of pollution-induced community tolerance is linked to structural and functional resilience of a soil bacterial community following a five-year field exposure to copper. Soil Biol. Biochem. 42, 748–757.

Campillo-Cora, C., Soto-Gómez, D., Arias-Estévez, M., Bååth, E., Fernández-Calviño, D., 2021. Bacterial community tolerance to Cu in soils with geochemical baseline concentrations (GBCs) of heavy metals: Importance for pollution-induced community tolerance (PICT) determinations using the leucine incorporation method. Soil Biol. Biochem. 155, 108157.

Campillo-Cora, C., Soto-Gómez, D., Arias-Estévez, M., Bååth, E., Fernández-Calviño, D., 2022. 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. Biol. Fertil. Soils 58, 49–61.

Fernández-Calviño, D., Bååth, E., 2016. Interaction between pH and Cu toxicity on fungal and bacterial performance in soil. Soil Biol. Biochem. 96, 20–29.

Hernandez-Soriano, M.C., Jimenez-Lopez, J.C., 2012. Effects of soil water content and organic matter addition on the speciation and bioavailability of heavy metals. Sci. Total Environ. 423, 55–61.

Imfeld, G., Bringel, F., Vuillemeumier, S., 2011. Bacterial tolerance in contaminated soils: potential of the PICT approach in microbial ecology. In: Amiard-Triquet, C., Rainbow, S., Roméo, M. (Eds.), Anonymous tolerance to Environmental Contaminants. CRC Press, New York, pp. 335–364.

Kotaś, J., Stasicka, Z., 2000. Chromium occurrence in the environment and methods of its speciation. Environ. Pollut. 107, 263–283.

Laborda, F., Górriz, M.P., Bolea, E., Castillo, J.R., 2007. Mobilization and speciation of chromium in compost: a methodological approach. Sci. Total Environ. 373, 383–390.

Lekfeldt, J.D.S., Magid, J., Holm, P.E., Nybroe, O., Brandt, K.K., 2014. Evaluation of the leucine incorporation technique for detection of pollution-induced community tolerance to copper in a long-term agricultural field trial with urban waste fertilizers. Environ. Pollut. 194, 78–85.

Liu, X., Zhang, S., Wu, W., Liu, H., 2007. Metal sorption on soils as affected by the dissolved organic matter in sewage sludge and the relative calculation of sewage sludge application. J. Hazard. Mater. 149, 399–407.

Meisner, A., Bååth, E., Rousk, J., 2013. Microbial growth responses upon rewetting soil dried for four days or one year. Soil Biol. Biochem. 66, 188–192.

Pradhan, A., Geraldes, P., Seena, S., Pascoal, C., Cássio, F., 2016. Humic acid can mitigate the toxicity of small copper oxide nanoparticles to microbial decomposers and leaf decomposition in streams. Freshw. Biol. 61, 2197–2210.

Refaey, Y., Jansen, B., El-Shater, A.-H., El-Haddad, A.-A., Kalbitz, K., 2014. The role of dissolved organic matter in adsorbing heavy metals in clay-rich soils. Vadose Zone J. 13, 7.

Schmitt, H., van Beelen, P., Tolls, J., Van Leeuwen, C.L., 2004. Pollution-induced community tolerance of soil microbial communities caused by the antibiotic sulfachloropyridazine. Environ. Sci. Technol. 38, 1148–1153.

Tlili, A., Bérard, A., Blanck, H., Bouchez, A., Cássio, F., Eriksson, K.M., Morin, S., Montuelle, B., Navarro, E., Pascoal, C., Pesce, S., Schmitt. Jansen, M., Behra, R.,, 2016. Pollution-induced community tolerance (PICT): towards an ecologically relevant risk assessment of chemicals in aquatic systems. Freshw. Biol. 61, 2141–2151.

Wittbrodt, P.R., Palmer, C.D., 1997. Reduction of Cr(VI) by soil humic acids. Eur. J. Soil Sci. 48, 151–162.