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A bacterium-based contact assay for evaluating the quality of solid samples–Results from an international ring-test

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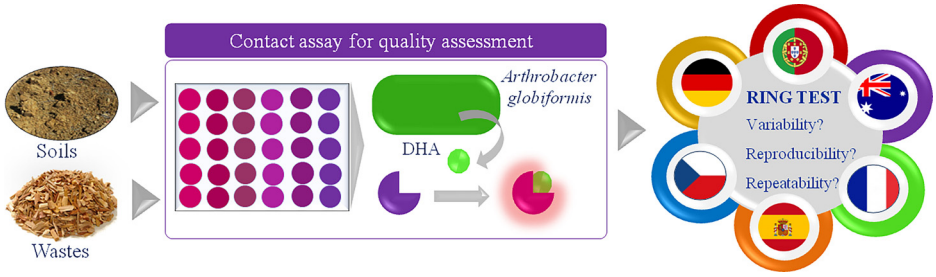
GRAPHICAL ABSTRACT



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ABSTRACT

The contact assay measuring the inhibition of *Arthrobacter globiformis* dehydrogenase activity as an endpoint to evaluate the toxicity of solid samples was tested in an international ring-test to validate its performance for ISO standardization (ISO/CD 18187). This work reports the results of the ring-test involving 9 laboratories from six countries. At least 8 valid data sets were obtained for each sample and more than three quarters of the participants attained the validity criteria defined in the standard. The coefficient of variation within (CV_r) and between (CV_R) laboratories was generally on average < 15% and < 30% for negative and positive controls, respectively. Regarding solid samples, the laboratories provided a similar ranking of the samples based on their toxicity, despite some variation in the LOEC values. The logarithmic within-lab standard deviation < 0.50 for soils and < 0.25 for wastes evidenced a good repeatability. The between-lab variability assessed by a CV_R < 30%, minimum-maximum factor < 4 and a reproducibility standard deviation (SD_R) < 0.13 for a great

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part of the solid samples, confirmed the test reproducibility. Overall, this assay proved to be robust, sensitive and feasible for routine use towards the quality assessment of soils and wastes.

1. Introduction

The quality assessment of soils and wastes has been recently receiving more attention, given the relevance of soil ecosystem services and their protection for different uses, as well as the need for a sustainable management of the increasingly produced solid wastes. Under this context, the evaluation of solid materials, which might be contaminated with a mixture of unknown compounds, has been broadening beyond the regular physical and chemical characterization as to include their ecotoxicological characterization [1]. This requires the development of new, easy-handled, cost-effective, rapid and sensitive methods to cover ecologically relevant terrestrial organisms that are yet to be considered for assessing the quality of solid samples. Soil microorganisms, for instance, are a key group in the terrestrial trophic chains, since they are responsible for a wide range of ecosystem functions, such as organic matter degradation, assimilation and dissimilation of N and plant-growth promotion [2–4]. Therefore, soil microorganisms have been often viewed as possible bioindicators of soil functioning and health [5,6]. Until now, most microbial parameters monitored in soils using ISO (International Organization for Standardization) standards are at the community level [7] like microbial diversity [8,9], biomass [10,11], metabolic activity (*e.g.*, enzymatic activities, respiration curves [12], ammonium oxidation [13], mineralization and nitrification [14]), and abundance of microbial genes [15].

Despite the relevance of these endpoints to estimate possible impacts of stressors in the composition and normal functioning of the soil microbial community, they may provide misleading sensitivity due to community shifts, namely associated with the disappearance of sensitive populations and the dominance of tolerant ones [16]. In addition, the methods used are expensive, time-consuming and effort-demanding. Thereby, the development of standard methods to quickly assess the quality of solid samples on specific microbial populations can be an excellent and reliable alternative. Indeed, such methods are not constrained by the functional redundancy occurring at microbial community level, besides being usually rather simple, cost-effective and easily standardized. As such, they are valuable tools to support risk assessment and hazard characterization schemes applied to soils [*e.g.*,17,18] and wastes [19], as often required by risk managers, industry and academics.

In particular, *Arthrobacter globiformis* is a ubiquitous and non-pathogenic aerobic soil bacterium that synthesizes an extracellular enzyme during different metabolic processes [20], which activity was suggested to be a potential indicator of the effect of contaminants on solid samples [21]. As such, a solid contact test based on the measurement of *A. globiformis* dehydrogenase activity (DHA) was preliminary purposed [21,22]. The principle of the assay relies on the reduction of resazurin into resorufin that is fluorimetrically detected and used as a proxy of DHA. Whenever a solid sample inhibits the *A. globiformis* DHA, the level of resorufin production and, hence, the emitted fluorescence is reduced, indicating that the sample is toxic or presents reduced quality. Several studies confirmed the bacterium sensitivity to different pollutants [*e.g.*,22–24], which was often more pronounced comparatively to other test methods and terrestrial test organisms [25,26]. Therefore, due to the short life-cycle, fast response, sensitivity and easy maintenance of *A. globiformis*, as well as the high surface-to-volume ratio and the requirement of small amounts of test sample, this solid contact assay was proposed for standardization to ISO.

A relevant step in the standardization process is the validation of the test procedures through an international ring-test (IRT) at the Committee stage (CD) to reach the Enquiry stage (DIS) [7]. Therefore, this work aims to evaluate the within- and between-laboratories variability of the method 'Solid contact test using the dehydrogenase activity of *Arthrobacter globiformis,* ISO/CD 18187′ [27], as requested by the ISO/TC 190/SC4 'Soil quality – Biological methods'. The IRT joined 9 laboratories from 6 countries to evaluate and validate the test method in what regards (i) its understandability and practicability, (ii) achievement of the validity criteria, (iii) suitability of the reference substance, (iv) sensitivity and responsiveness of the assay to different soil and waste samples, (v) repeatability of the assay, (vi) reproducibility and applicability of the assay for routine use in different

Table 1
Physical and chemical characterization and metal content of the soil and waste samples. *Data retrieved from ABANDA database [37].

	Soils				Wastes			
Test Items	S1	S2	S3	S4	W1	W2	W3	W4
Waste code	–	–	–	–	WOO	17 05 06	10 01 17	19 12 05
Description	Control soil	Construction site	Uranium mine	Phosphogypsum deploying site	Cu-treated Wood	Dredged harbor material	Fluidized bed ash	Crushed glass material
pH-value	6.23	6.93	4.25	4.14	5.18	6.89 *	9.96 *	9.06 *
Conductivity (μS cm^{-1})	271	159	293	2723	n.d.	n.d.	n.d.	n.d.
OM content (%)	2.93	4.20	2.36	5.39	46.4	3.91	6.54	1.04
Texture	Silty clay	Sandy loam	Loamy sand	Sandy loam	n.d.	n.d.	n.d.	n.d.
Sand (%)	9.9	55.2	85.9	69.1				
Silt/clay (%)	99.1	44.8	14.1	24.9				
Metals (mg Kg^{-1})								
Cu	3.06	24.32	22.83	11.46	1057.31	92.7	254.1*	5.69*
Pb	1.36	35.47	32.01	20.88	14.30	162.2	2187*	148.2*
Cd	0.38	0.34	21.81	0.11	0.27	2.1	8*	0.76*
Cr	8.67	14.71	33.36	9.86	298.16	171.8	n.d.	3.37*
U	< dl	< dl	169.2	1.63	n.d.	n.d.	n.d.	n.d.
Zn	50.72	155.15	118.83	89.51	43.50	582.2	4049*	1222.09*
Fe	742.37	1631.71	2413.54	10870.4	n.d.	n.d.	n.d.	n.d.
Ni	1.88	6.36	32.96	17	n.d.	n.d.	n.d.	n.d.
As	n.d.	n.d.	n.d.	n.d.	n.d.	52.5	99.8*	n.d.
PAH (μg Kg^{-1})	n.d.	8.5	n.d.	n.d.	n.d.	6.1	n.d.	n.d.

* dl – detection limit; n.d. – not determined.

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