Geoderma 263 (2016) 132–139

Contents lists available at ScienceDirect

Geoderma

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

# Is soil basal respiration a good indicator of soil pollution?

A. Romero-Freire *, M. Sierra Aragón, F.J. Martínez Garzón, F.J. Martín Peinado

*Department of Soil Science, University of Granada, Campus Fuentenueva, 18002 Granada, Spain*

A R T I C L E I N F O

*Article history:*
Received 31 January 2015
Received in revised form 27 March 2015
Accepted 14 September 2015
Available online 29 September 2015

*Key words:*
Soil properties
Soil basal respiration
Soil pollution
Toxicity
Metal(loid)s

A B S T R A C T

Metal(loid)s are common pollutants in soils, causing a significant toxicological risk to living organisms and to the ecosystems. Soil basal respiration (SBR) is broadly used as indicator of metal(loid) stress in polluted soils, although the correlation with toxicity gives in many cases contradictory results. In this paper, we study seven different soils with contrasting properties and with different pollution levels of As, Pb, Zn, and Cu to assess the influence of soil properties and contaminant concentration in the SBR response. In general terms, the SBR showed toxic effects in soils with low organic-matter content and acidic pH values. Low respiration rates were found in soils polluted with As even at very high contamination levels. According to our results, SBR is not a good indicator of pollution by Pb, Zn and Cu in soils rich in organic carbon or in highly carbonate soils. In As-polluted soils, SBR also showed a low sensitivity in all cases. Further studies are needed to assess the role of soil properties and the type of pollutant in the SBR tests.



## 1. Introduction

Soil pollution can reduce microbial biomass (Shukurov et al., 2014), affect the taxonomic diversity of soil communities (Stefanowicz et al., 2008), and it may act on a variety of microbial processes in the soil, thereby disturbing the nutrient cycling and the capacity to perform key ecological functions, such as mineralization of organic compounds and synthesis of organic substances (Giller et al., 1998; Moreno et al., 2009). Metals and metalloids do not degrade and may accumulate in soils and sediment (van Gestel, 2008), becoming an environmental concern due to their effects on ecological functions on soils. Metal(loid)s such as As, Pb, Zn or Cu, that reach the soil from different anthropogenic activities (industry, mining, smelters, agriculture, etc.), are common pollutants in soil contamination with a serious potential to degrade soil ecosystems (Burgos et al., 2008), and can pose significant toxicological risks to organisms (Song et al., 2009).

Microbial communities constitute one of the most suitable groups to study soil degradation, as they are ubiquitous and respond quickly to changing conditions (Nannipieri et al., 2003). In addition, it has been suggested that they should be included in ecological-risk assessments as key endpoints to follow the toxicity through time (White et al., 1998; Frey et al., 2006). Assessments of metal(loid) effects on SBR require a range of parameters to be measured, related both to chemical properties of the pollutants as well as to soil properties, particularly when the study involves different soils with contrasting properties that can strongly affect the soil-respiration response (Khan and Scullion, 2002). Some authors have observed that the main soil properties which influence soil respiration are clay content (Wang et al., 2013), organic carbon content (Balogh et al., 2011), nitrogen content (Lin et al., 2010; Ramirez et al., 2010), pH and carbonate compounds (Stefanowicz et al., 2008; Azarbad et al., 2013). Moreover, soil properties also influence the water solubility and bioavailability of metal(loid)s in soils and therefore, they can modulate the effect of potential pollutants on microbial activity and microbial community composition. In this way, soil respiration has been studied and extensively described as a biochemical process that also depends on physical properties, indicating that soil–water content and temperature are the physical parameters that explain part of the soil-respiration variance (Lloyd and Taylor, 1994; Fang and Moncrieff, 2001; Chen et al., 2010; Balogh et al., 2011; Wang et al., 2014).

Soil basal respiration (SBR) of microbial biomass is a major attribute related to soil fertility (Niemeyer et al., 2012) and a common indicator of soil quality (ISO, International Organization for Standardization, 2002). The presence of pollutant elements in soils can significantly hamper the ability of bacteria to decompose complex substrates (Burkhardt et al., 1993; Nwachukwu and Pulford, 2011), and therefore the amount of $CO_2$ produced is a reliable index of the effect of metal(loid) contamination on microbial activity (Rost et al., 2001; Nwachukwu and Pulford, 2011; Kaplan et al., 2014). Therefore, respirometry measurements are valuable indicators of soil quality and might signal metal(loid) stress to soil microorganisms (Azarbad et al., 2013; Dai et al., 2004). However, soils are systems with a great complexity and the behaviour of microorganisms to contamination may be very variable, raising doubts on the possibility of use respirometry responses as reliable indicators of soil contamination. Thus, some authors found no evidences in the decrease of soil respiration with the increase of the pollution (Wakelin et al., 2010; Zornoza et al., 2015); moreover, other authors observed that contaminated soils presented higher respiratory activity than unpolluted

* Corresponding author at: Campus Fuentenueva s/n, 18002 Granada, Spain.
*E-mail address:* anaromerof@ugr.es (A. Romero-Freire).

http://dx.doi.org/10.1016/j.geoderma.2015.09.006
0016-7061/

ones (Scelza et al., 2008) and that respiration rates can augment when the soil contamination level increases (Dinesh et al., 2012). These differences in SBR response in relation to pollution can reflect changes in soil microbial community composition (e.g. changes in the relative proportions of fungi over bacteria), showing the resistance and resilience of soil microbial communities to certain types of contamination (Scelza et al., 2008; Allison and Martiny, 2008; Hänsch and Emmerling, 2010).

The contradictory results listed above, lead us to provide in this manuscript new information in relation to the comparison between soil toxicity by metal(loid)s and soil basal respiration. We combined soil characterization, chemical extractions and SBR to unravel the link between the effects of contaminants and soil physicochemical properties, with the aim to investigate the effect of pollution by some of the most common trace metal(loid)s in soils (As, Pb, Zn, and Cu) on the soil microorganism activity, estimated from soil basal respiration (SBR). We focused on studying the influence of soil properties and constituents in the potential toxicity of metal(loid)s and in the role of SBR in the assessment of soil contamination.

## 2. Material and methods

### 2.1. Soil sampling and characterization

Seven soil horizons (H1–H7) with different properties, from the main soil groups in Spain were selected (Table 1). All these uncontaminated soils were collected in the field and cleared of stones and plants. Afterwards, the soils were dried in a thin layer at 25 °C and then sieved through 2 mm. The main parameters analysed were: pH (soil:0.1 M KCl, ratio 1:2.5); texture (Loveland and Whalley, 1991); nutrients, bases, and cation-exchange capacity (CEC) (USDA Soil Conservation Service, 1972); organic carbon (OC) (Tyurin, 1951); water-holding capacity (WHC) (ISO, International Organization for Standardisation, 1996); available water (AW) (Richards, 1945) and calcium carbonate content ($CaCO_3$) (Barahona and Iriarte, 1984). Moreover, the amorphous forms of iron, aluminium, and manganese oxides were analysed according to Holmgren (1967) and Schwertmann and Taylor (1977), respectively.

### 2.2. Soil contamination

Soil samples were individually spiked in the laboratory with the metal(loid)s from solutions of soluble salts of the most abundant chemical species present in the soils: As(V) [$Na_2HAsO_4$ $7H_2O$], Pb(II) [$Pb(NO_3)_2$], Cu(II) [$Cu(NO_3)_2$ $3H_2O$], and Zn(II) [$ZnCl_2$]. Pollution levels were established by increasing the background concentrations of the soils according to the intervention values proposed by the Regional Government of Andalusia (Aguilar et al., 1999). Five contamination levels were defined: L1 (intervention level for agricultural soil), L2 (intervention level for natural areas), L3 (intervention level for industrial areas), L4 (L3 × 2), and L5 (L3 × 4 in the case of As, Pb, Cu, and L3 × 3 in the case of Zn) (Table 2). In all cases, uncontaminated soil samples were used as a control (L0), making a total of 6 treatments for each studied soil. A total of 126 experimental units (7 soils × 6 treatments × 3 repetitions) were used in this study for each pollutant element.

The contamination was made by spiking 50 g of soil with the individual pollutant, and the moisture was brought to 60% of their water-holding capacity. Soils were incubated for 4 weeks at 25 ± 1 °C and 60% air humidity, with a light cycle of 10 h. The water content of each sample was checked and corrected weekly, maintaining the incubation conditions constant so as not to disturb the microbial activity. The incubation period was chosen to stabilize the contaminant added, optimising the time spent on these tests, and was selected based on similar studies by other authors (Fendorf et al., 2004; Tang et al., 2006; Martín Peinado et al., 2012).

### 2.3. Metal(loid) analysis

Total trace metal(loid) concentration (mT) in soils was determined from acid digestion in strong acids ($HNO_3$ + HF). Water-soluble forms (mW) were determined from soil:water extracts (1:1 ratio) obtained by shaking for 24 h and extracted with 10 cm Rhizon MOM (Metal and Organic Matter) soil moisture samplers. In all cases, trace metals were measured by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) in a spectrometer ICP-MS NEXION 300D. For calibration, two sets of multi-element standards containing all the analytes of interest at five different levels of concentration were prepared using rhodium as the internal standard. All standards were prepared from ICP single-element standard solutions (Merck, Darmstadt, Germany) after dilution with 10% $HNO_3$. Procedural blanks for estimating the detection limits ($3 * \sigma$; n = 6) were <0.21 ppb for As, <0.23 ppb for Pb, <2.68 ppb for Zn, and <0.52 ppb for Cu. The analytical precision was better than ±5% in all cases. The accuracy of the method was confirmed by analysing Standard Reference Material SRM2711 Montana Soil (US NIST, 2003) (n = 6). For As, Pb, Zn, and Cu the average recovery values ranged between 91% and 105% of the certified reference values.

### 2.4. Soil respiration

Polluted soils were incubated for 4 weeks at 60% of their water-holding capacity. The basal respiration rate ($R_B$), based on ISO,

**Table 1**
Mean values and standard deviation (±*SD*) of the main properties of selected samples.

| Sample | Soil horizon | pH (KCl) | AW (%) | $CaCO_3$ (%) | OC (%) | N (%) | P (mg $kg^{-1}$) | Clay (%) | CEC ($cmol_+$ $kg^{-1}$) | $Fe_o$ (g $kg^{-1}$) | $Mn_o$ (g $kg^{-1}$) | $Al_o$ (g $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| H1 | Ah | 7.63 (±*0.02*) | 8.08 (±*0.23*) | 37.11 (±*0.44*) | 5.43 (±*0.38*) | 0.35 (±*0.03*) | 8.26 (±*0.81*) | 23.61 (±*0.90*) | 21.43 (±*2.00*) | 0.68 (±*0.03*) | 0.06 (±*0.01*) | 1.17 (±*0.03*) |
| H2 | Bw | 8.11 (±*0.09*) | 5.72 (±*0.41*) | 72.39 (±*0.86*) | 0.42 (±*0.12*) | 0.02 (±*0.01*) | bdl (–) | 11.79 (±*0.44*) | 9.83 (±*1.00*) | 0.20 (±*0.02*) | 0.02 (±*0.01*) | 0.31 (±*0.02*) |
| H3 | Ck | 8.24 (±*0.09*) | 5.37 (±*0.07*) | 92.32 (±*1.80*) | 0.38 (±*0.17*) | 0.03 (±*0.01*) | bdl (–) | 7.70 (±*0.58*) | 2.94 (±*0.13*) | bdl (–) | bdl (–) | 0.15 (±*0.02*) |
| H4 | Bw | 5.80 (±*0.21*) | 6.60 (±*0.18*) | nd (–) | 0.61 (±*0.10*) | 0.05 (±*0.02*) | 6.53 (±*0.65*) | 19.05 (±*0.38*) | 9.91 (±*0.42*) | 0.52 (±*0.09*) | 0.18 (±*0.02*) | 0.38 (±*0.07*) |
| H5 | Ah | 6.72 (±*0.06*) | 12.53 (±*2.09*) | nd (–) | 8.22 (±*0.02*) | 0.44 (±*0.01*) | 28.06 (±*0.69*) | 23.79 (±*0.12*) | 25.90 (±*0.37*) | 0.65 (±*0.14*) | 0.41 (±*0.07*) | 0.50 (±*0.06*) |
| H6 | C | 4.58 (±*0.06*) | 7.40 (±*0.04*) | nd (–) | 0.49 (±*0.02*) | 0.44 (±*0.02*) | 1.09 (±*0.11*) | 8.31 (±*0.12*) | 3.83 (±*0.37*) | 1.00 (±*0.06*) | 0.09 (±*0.01*) | 0.27 (±*0.06*) |
| H7 | Bt | 5.86 (±*0.01*) | 5.49 (±*0.21*) | 0.92 (±*0.16*) | 0.66 (±*0.25*) | 0.04 (±*0.02*) | bdl (–) | 54.76 (±*1.13*) | 15.53 (±*1.01*) | 0.78 (±*0.07*) | 0.03 (±*0.01*) | 0.73 (±*0.06*) |

bdl: below detection limit; AW: available water; $CaCO_3$: calcium carbonate content; OC: organic carbon content; CEC: cation exchange capacity; Feo/Mno/Alo: amorphous oxides forms; nd: not detected.