Ecological Indicators 75 (2017) 118–125

Contents lists available at ScienceDirect

Ecological Indicators

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

Application of stress index in evaluating toxicological response of soil microbial community to contaminants in soils

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ARTICLE INFO

Article history:
Received 16 May 2016
Received in revised form
28 November 2016
Accepted 2 December 2016

Keywords:
Soil contamination
Ecological risk assessment
Siduron
Microbial stress index

ABSTRACT

Toxic effects of chemical contaminants on soil microbial community structure and function have been widely studied. However, it is difficult to assess risks regarding soil microbial toxicity. In the ratio to reference approach, the stress index (SI) is used to indicate the relative change of biological response of organisms compared to a reference. In this study, the SI approach was used to assess the soil microbial stress levels of multiple heavy metal contaminated urban soil in three sites. Soil microbial community functional parameters suggested that two heavy metal contaminated sites (G and D) had apparently higher stress indexes relative to the reference site, N. Use of those similar microbial community functional parameters revealed that parameters such as N and C mineralization and alkaline phosphatase activity were sensitive to the addition of the herbicide Siduron. Soil G, which had the highest microbial stress index, showed more severe impairment when amended with Siduron, while soil N and soil D showed dose-dependent responses to Siduron. Overall, the results of this study indicated that, when compared with direct use of real data describing microbial parameters, use of SI in ecological risk assessment is more indicative and helpful.

1. Introduction

In ecological risk assessment, the relationship between extra stress and biological response is critical to quantifying hazards. In the triad risk assessment approach, a lower tier is always aimed at identifying potential adverse effects of contaminants. Toxic effects of chemical pollutants such as pesticides and heavy metals on soil microbial community structure and function have been widely studied. A general reduction in the activity of soil enzymes including ß-glucosidase, ß-xylosidase, cellobiohydrolase, and phosphatase was observed with increasing levels of the herbicide nicosulfuron in soils (Karpouzas et al., 2014). Thus, contaminant-sensitive microbial parameters can be used in the lower tier to identify damage. To date, there have been many ISO standardized methods developed in soil microbiology to test the structure and function of the soil microbial community (Philippot et al., 2012). However, risk assessment of pollutants for their soil microbial toxicity has seldom been reported. One of the critical reasons for this is lack of practical ways to estimate the effects.

Field application of microbiological parameters is limited because of complicated integrated impacts from various environment conditions. As occurred in application of biomarkers, a simple method for solving this problem is to summarize microbiological responses and simplify their interpretation during risk assessment. The ratio to reference approach is commonly applied in ecological risk assessment. In this method, the risk of contamination is quantified by comparing the responses of organisms at ecotoxicological and ecological levels to those from a reference site, which was first suggested in application of biomarkers (Dagnino et al., 2008; Semenzin et al., 2008). During application of this method, it should be assumed that there is a reference site that has similar physical and chemical properties as the soils at the study sites.

In toxicology, stress is defined as a measurable alteration at the biochemical and/or physiological levels caused by a change in the environment that would result in a reduction of adaptation to further adverse conditions (Bayne, 1986). Here, we considered the response of the soil microbial community to the stress of contaminated soil. Relative stress responses (RSR) are calculated by comparing the responses of microbial organisms exposed to contaminated soil with those exposed to the reference soil. The RSR is

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http://dx.doi.org/10.1016/j.ecolind.2016.12.002
1470-160X/

then compared with two threshold values (Th 1 and Th 2) (Dagnino et al., 2008). Th1 is the test result below which the impairment level for the tested endpoint can be regarded as negligible and/or caused by natural variability, while Th2 is the test results above which the impairment level for the tested endpoint is regarded to be no more significant (Semenzin et al., 2008). The default Th1 value usually corresponds to a variation of 20% of the reference data (Chapman et al., 2002; Dagnino et al., 2008), while default Th2 values have been arbitrarily determined by experts. Dagnino et al. (2008) suggested that a Th2 default value corresponded to a variation of 100% for increasing stress response profiles and 80% for decreasing stress response profiles. By comparing Th1 and Th2, a stress index (SI) is estimated for each RSR. Thus, SI is a standardized value that ranges from 0 to 1 as the stress increases from none to highest. As shown in Table 1, the dimensionless SI value could be classified into five impairment classes (Semenzin et al., 2008). In that way, the effects of contaminants can be easily assessed.

Toxic effect tests of contaminants on organisms are usually conducted in spiked soil or other matrices such as filter paper. Toxic responses at a range of concentration gradients of contaminants are recorded. Dose-dependent effects of contaminants are estimated by the quantitative relationship between responses and concentration gradients. However, the changes in responses at biological levels are always unstable and the quantitative dose-effect relationship between stress and bio-response is also uncertain from one case to another, which is one of the reasons for the large gap between lab and field results. It could be more objective to assess effects of contaminants using the ratio to reference approach. The SI value obtained by comparison of Th1 and Th2 represents the risk caused by contaminants after removing the variation associated with natural conditions. Thus, one of the benefits of the ratio to reference approach is that it is convenient to quantify stress.

Urban soil in some parks in Beijing has been contaminated with multiple heavy metals for a long time due to anthropogenic disturbances (Wang et al., 2012). Siduron is a pre-emergence herbicide used in north China to combat crabgrass and grassy weeds in urban lawns. Herbicides reaching the soil in significant quantities have direct effects on soil microbiological aspects. However, few studies have investigated the ecological health or functions of urban soils in Beijing, despite the many reports of accumulation and distribution of heavy metals in urban soils (Wang et al., 2011). The integrated stress index is usually more useful to visualize between-site and/or between survey differences for comparison with exposure (Dagnino et al., 2007). In this study, the integrated stress index known as the microbial stress index (MSI) was evaluated in urban park sites in which soils were contaminated with multiple heavy metals. The toxic stress of the herbicide Siduron on microorganisms and their processes in soils of those sites were also estimated using SI and MSI. The purpose of this study was to test the applicability of SI to ecological risk and ecotoxicological stress assessment, to reveal the soil microbial community health and functions of sites contaminated by multiple heavy metals in urban parks, and to predict the toxic stress of Siduron on microorganisms and processes in urban contaminated soils in Beijing.

Table 1
Impairment classes based on stress index with linguistic evaluation and colors (Semenzin et al., 2008).

Stress index (SI)	Linguistic evaluation (Symbol)	Color
0.0	Negligible (N)	White green
$0.0 < SI \leq 0.3$	Intermediate I (I)	Green
$0.3 < SI \leq 0.7$	Intermediate II (II)	Yellow
$0.7 < SI < 1.0$	Intermediate III (III)	Orange
1.0	Relevant (R)	Red

2. Materials and methods

2.1. Study sites soil sampling

The study sites (N, D, G) were located in a public park, Nanguan Park. All three sites are covered by lawns and garden shrubs. Topsoil (0–20 cm) was sampled after removing the covering plants and roots. One composite sample from five sub-sites within a 10 m × 10 m square was collected from each site. For microbiological tests, fresh soil samples were sieved to pass through a 2-mm screen, then stored in the refrigerator at 4 °C. All tests were conducted within one week of sampling.

2.2. Chemical and biological analysis

Heavy metal concentrations (Cu, Cd, Pb, Zn) in soils were determined by a four-acid digestion method. Namely, aliquots of 0.25 g ground soil (0.149 mm sieved) were dissolved in a mixture of 10 ml HCl, 5 ml HNO_3, 5 ml HF, and 3 ml $HClO_4$ and digested on hotpots. The digested extracts were then diluted to 50 ml with deionized water for subsequent measurement of Zn using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The solution was further diluted to 250 ml for determination of Cu, Cd and Pb using inductively coupled plasma mass spectrometry (ICP-MS).

Soil pH was determined in a 1:2.5 (w/v) soil and water suspension after shaking the mixture for 30 min using glass electrodes potentiometry. Soil texture was determined by a laser particle size analyzer and the outcomes were reported according to the USDA soil classification scheme, in which the clay content (CLAY) denoted the percentage of particles with sizes less than 0.002 mm. The soil organic carbon (SOC) content was measured using a C-N-S elemental analyzer after the soil aliquot was treated with 1 mol/L HCL to remove the inorganic carbon.

Soil biological analysis was conducted using fresh soil samples. Soil microbial biomass carbon (MBC) was determined using the fumigation–extraction technique. Total C extracted using K_2SO_4 solution was determined using a TOC analyzer (Liqui TOC), after which the total C was transformed to C_{mic} using factor k_e C = 0.35.

N mineralization potential was determined over a 4-week period at 35 °C using incubation intervals of 1, 2, 3 and 4 weeks. Mineral N was extracted from the soils before the first incubation and following each of three incubations using 0.01 M $CaCl_2$ and a minus-N nutrient solution (Stanford and Smith, 1972).

Carbon mineralization was determined based on the amount of CO_2 released by soil incubated at 28 °C for 35 days. Five milliliters of 0.5 M NaOH were incubated together with the soil to absorb the released CO_2. The amount of CO_2 absorbed was determined by titrating the NaOH solution with 1 M HCl.

Activities of urease (pH 7.4), arylsulphatase (pH 5.8), alkaline phosphatase (pH 11), and invertase (pH 4.5) were assayed by incubating an aliquot of soil with a substrate of the reaction for a given period of time. After incubation, the absorbance values of metabolites of urease, arylsulphatase, alkaline phosphatase and invertase were determined at 578, 400, 578 and 508 nm, respectively (Gosewinkel and Broadbent, 1984; Tabatabai and Bremner, 1970; Kramer and Yerdei, 1959; Frankeberger and Johanson, 1983).

Substrate utilization patterns of soil microbial communities were analyzed using ECO microplates. Briefly, 125 μl of diluted soil suspensions for each well of the ECO plates were incubated at room temperature and the absorbance (590 nm and 720 nm) of the 96 wells was recorded in 24 h intervals over 144 h. An incubation of 96 h was selected for the final analysis of the biological test (Dobler et al., 2001). The color intensity is a measure of the metabolic activity of a community, which is expressed for each soil sample based on the average well color development (AWCD), calculated from