Changes in enzyme activity and functional diversity in soil induced by Cd and glucose addition

A R Gilmullina, P Yu Galitskaya and S Yu Selivanovskaya

Institute of Environmental Sciences, Kazan Federal University, Kremlevskaya str., 18, 420008, Russia

E-mail: gilmullinaar@mail.ru

Abstract. Toxic heavy metal (HM) contamination is a major global issue as it may have an indirect effect on the health of soil, plants, animals and, consequently, on human health. Agricultural soils’ fertilization is one of the reported sources of HM pollution in the world. In this case simultaneous input of stimulating and inhibiting agents into soil takes place, and effects of the combined influence of these agents is hardly predictable. In this study, a simultaneous inhibiting and stimulating effect of Cd and glucose on soil microbes was studied in a model experiment. Enzyme activities (phosphatase, β-glucosidase and cellobiohydrolase) and functional diversity \textit{(BIOLoG\textsuperscript{®} EcoPlates\textsuperscript{TM})} were assessed as a test functions. Cd (300 µg Cd g\textsuperscript{-1}) amendment had a negative effect only on phosphatase activity. Glucose (3 mg C g\textsuperscript{-1}) addition inhibited β-glucosidase activity and stimulated functional diversity. In joint addition of Cd and Glucose the leading effect belonged to that agent which had the greatest effect in case when it was added separately.

1. Introduction

Toxic heavy metal (HM) contamination is a major global issue as it may have an indirect effect on the health of soil, plants, animals and, consequently, on human health [1 - 3]. Cadmium (Cd) is one of the most toxic metals because it can be easily transferred through food web [4]. Generally, Cd is distributed at concentration between 0.1 and 1.0 mg kg\textsuperscript{-1} in different soils. The physicochemical properties of soils can influence the concentration of Cd [1]. For example, acid pH can improve Cd mobility [5] and clay particles decrease Cd mobility by colloids formation [6].

Several studies investigated that HM contamination may occur from different kinds of anthropogenic activity such as industrial wastes disposal, car emissions and agricultural treatments [7 - 9]. The main HM contamination sources occurring due to agricultural treatment are the use of composted waste and sewage sludge as fertilizers [10] These fertilizers contain available organic matter and the amendment is known to be enough good to improve soil fertility. However, such treatment may also contribute HM contamination inhibiting soil microbial community [11].

Soil biological activity reflects the status of soil microbial community. Soil microorganisms take part in nutrient cycling and the decomposition of organic matter. Any soil disturbance may cause the changes in diversity and activity of soil microbial population [12]. Activity of soil microbial population may be estimated by different parameters such as respiration, soil enzymes, community level physiological profiling etc. Soil enzymes are microbial metabolites, therefore, enzyme activity measurement allows to indirectly track the changes in carbon (C), nitrogen (N) and phosphorous (P) cycle in soil. Biolog Ecoplate contains 31 different C-containing sources (aminoacids, carbohydrates and carboxylic acids). This technique is based on measuring oxidation ability of the C substrates to
create pattern on potential sole C source utilisation [13]. So, application of this method allows to estimate soil microbial community involved in C cycling [14].

HM have different effect on soil microbial activity and the effect mainly depends on its concentration and soil parameters. There are several studies which have already demonstrated various effects of HM on phosphatase, urease, dehydrogenase activity and functional diversity of microbial communities [7, 14, 15]. Also investigations concerning the effects of organic compounds on soil microbial communities are presented [1, 11]. However, the effect of simultaneous inhibition and stimulation remains unclear.

Thereby, present study aimed to investigate combined effect of HM and organic matter on enzyme activity and functional diversity of microbial communities in the soil with different physical properties taking into account the individual effects of inhibition and stimulation agents.

2. Material and methods

Soil samples were sampled from three different depths (0-20 cm; 20-40 cm; 40-60 cm) in temperate mixed forest located at Laishevo district, Republic of Tatarstan (Russia). Soil was classified as sandy loamy luvisol. Soil initial parameters are shown on Table 1.

Table 1. Soil physiochemical properties.

| Parameter | Soil sample |
|-----------|-------------|
| Corg, %   | s0-20 | s20-40 | s40-60 |
| DOC, mg g\(^{-1}\) | 0.0162 | 0.0077 | 0.0016 |
| pH        | 5.8   | 5     | 5.1   |
| moisture, % | 21.1  | 4.8   | 6.5   |
| sand, %   | 54.5  | 81.3  | 85.1  |
| silt, %   | 41.2  | 18.5  | 14.1  |
| clay, %   | 4.2   | 0.1   | 0.7   |
| Cmic, mg g\(^{-1}\) | 0.301 | 0.169 | 0.011 |

Prior to experiment soil samples were moistened up to 40 % of WHC (water holding capacity) and pre-incubated at 23\(^{\circ}\)C for two weeks. Then soil samples were treated with water solutions containing 3 mg C g\(^{-1}\) glucose, 300 µg Cd g\(^{-1}\) CdCl\(_2\), mix of 3 mg C g\(^{-1}\) glucose and 300 µg Cd g\(^{-1}\) CdCl\(_2\). Final addition of water solutions adjusted the moisture of soil samples to 70 % of WHC. Soil samples treated only with water were used as control samples. Treated soils were incubated for 14 days at 23°C and samples were taken at the 1\(^{\text{st}}\), 3\(^{\text{rd}}\), 7\(^{\text{th}}\) and 14\(^{\text{th}}\) days for estimation of physiological activity of soil microbial community which was measured as enzyme activity and functional diversity. For these analyses soil samples were suspended in water (1:10).

Enzyme activity was estimated using 4-methylumbelliferone (MUF) containing substrates: MUF-β-d-cellobioside MUF for cellobiohydrolase, MUF-β-d-glucopyranoside for β-glucosidase and 4-MUF-phosphate for phosphatase. 50 µl of soil extracts and 50 µl of 200 µM MUF-containing substrate were transferred into black microplates. The fluorescence intensity was measured at an excitation wavelength of 355nm and an emission wavelength of 460 nm by microplate reader (Fluoroskan Ascent \(^{\text{\textregistered}}\)).

Functional diversity was determined using BIOLOG® EcoPlates \(^{\text{\textregistered}}\). Soil extracts were diluted by 10\(^{-4}\) and then transferred to microplates. The plate contains 31 different organic substrates with tetrazolium redox dye. When the substrate is oxidised the colour of well is changed which is measured by spectrophotometer (Multiskan FS, 595 nm). Colour change was measured every day during 13 days of incubation.
The microbial response was estimated using the next indices: average well colour development (AWCD), functional richness ($R$) and Shannon index ($H$). AWCD was calculated as follows:

$$AWCD = \frac{1}{n} \sum (A_i - A_0).$$

where $A_i$ is optical density of well $i$, $A_0$ is optical density of blank well.

Functional richness ($R$) was calculated as total number of wells with optical density higher than 0.25. Functional diversity was estimated by calculation of $H$ according to:

$$H = -\sum p_i \ln(p_i)$$

where $p_i$ is the proportional colour change in each well with substrate to the sum of colour change of all wells with substrate.

3. Results and discussion

Soil enzymes are known to be sensitive indicators of soil health. The assessment of enzyme activity gives information about the intensity of soil processes. Enzyme activity was 10-50 times higher in topsoil (0-10 cm) compared to subsoil samples (20-40 cm and 40-60 cm) in all treatments and all sampling days (Figure 1). Besides, enzyme activity did not differ significantly in subsoil samples regardless of different treatments.

**Figure 1.** The effect of Cd and glucose on enzyme activity: (a) phosphatase, (b) $\beta$-glucosidase and (c) cellobiohydrolase in different top- and subsoil layers during 2 weeks of incubation. Vertical bars demonstrate SE (n=3).

Phosphatase activity was the highest among other enzyme activities and was sensitive to Cd addition (Figure 1a). Cadmium addition inhibited phosphatase activity by 286-297 % in the both of Cd-contained topsoil treatments (+/- Glucose) at the 1st day of incubation. By the incubation time inhibiting effect of Cd slightly diminished but remained high (137-256 %).

$C$-cycle enzymes were presented by $\beta$-glucosidase and cellobiohydrolase which are involved in cellulose degradation. $\beta$-glucosidase activity was inhibited by 60-120 % in topsoil samples amended with glucose after the 1st day of incubation (Figure 1b). This might occur due to inhibition by product as $\beta$-glucosidase hydrolyses cellobiose releasing glucose molecules. But then at the 3rd day of incubation $\beta$-glucosidase activity rose to control value and remained at the same level up to the end of experiment. Cellobiohydrolase activity also decreased after glucose amendment at the 1st day of incubation (Figure 1c). However, cellobiohydrolase activity was several-fold lower in comparison to $\beta$-glucosidase and phosphatase activity.
Biolog Ecoplates were used for assessment of functional diversity of soil microbial community. This microplate system contains 31 C substrates which are predominantly amino acids, carbohydrates and carboxylic acids. The technique based on the measurement of substrate oxidation which gives the picture of functional diversity, i.e. the pattern of sole C source oxidation. The data is usually presented by calculation of different indices.

Figure 2 presents the data of calculated indices: average level of C source utilisation (AWCD), number of oxidised substrates (R) and functional diversity based on variety of oxidised substrates (H). Physiological activity was higher in topsoil samples compared to subsoil samples. Besides, both of subsoil samples were almost similar to each other and there was no effect of both of agents (Glucose/Cd) on the calculated indices in subsoil.

**Figure 2.** The effect of Cd and glucose on functional diversity: (a) average well colour development (AWCD), (b) Richness (R) and (c) Shannon index (H) in different top- and subsoil layers during 2 weeks of incubation. Vertical bars demonstrate SE (n=3).

AWCD, R and H were significantly higher in the treatments amended with glucose. Calculated indices (AWCD, R and H) started to increase only after the 3rd day of incubation in soil treated with glucose (glu-20). The interesting point is that samples treated with Glucose and Cd (Cdglu0-20) responded very quickly and were characterised by increase of AWCD (150 %), R (50 %) and H (80 %) even at the 1st day of incubation remaining at the same level for all incubation time. The highest values of calculated indices were observed for Cdglu0-20 at the 14th day of incubation.

In this study Cd amendment had a negative effect only on phosphatase activity which is in agreement with finding of other studies [1, 8]. But there was no effect of Cd on C-cycle enzymes and functional diversity. Contrary to our finding, some studies demonstrated the decrease of β-glucosidase activity [1, 7] and functional diversity [13, 14] after HM treatment during long-term experiments. In our case the experiment was planned as short-term, so we suppose that these parameters respond to HM treatment with delay. Sole glucose affected on β-glucosidase activity which can be explained by product inhibition. Glucose treatment also increased functional diversity and this is in agreement with studies which examined the effect of different stimulating agents (organic and inorganic fertilisers) on microbial activity [16, 17]. Additionally, it is necessary to note that the increase of physiological activity in sample treated with Cd and Glucose was quicker and higher than in sample treated only with glucose. This demonstrates that in case of sole glucose addition microorganisms competed for easily available C source at the same time regulating their population. In contrast, joint addition of Cd and glucose permitted to develop Cd-tolerant population without any competition.

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