Soil enzymes as biodiagnostics indicator of heavy metal pollution of urbanozem

E I Novosyolova, O O Volkova and R R Turyanova

Biological faculty, Bashkir State University, Street Zaki Validi, 32, 450076, Russia
e-mail: novoselova58@mail.ru

Abstract. The article presents a comparative analysis of the impact of the introduction of different doses of copper and cadmium on the activity of redox enzymes of urbanozem, collected from different territories of Ufa. The studies established the inverse relationship of the activity of catalase and polyphenol oxidase, and the direct one of the activity of peroxidase that depends on the doses of heavy metals, that allows to recommend their use as bioindicator of pollution of urbanozem with these metals. The reaction of the studied enzymes on the introduction of heavy metals is an indicator of their toxicity to living things at the molecular level. Comparative analysis of the impact of cadmium and copper in different doses on the activity of soil enzymes did not reveal a uniform regularity. Each of the metals showed their toxicity in different ways depending on the duration of their impact.

1. Introduction

Destructive anthropogenic pressure on soil cover in the cities led to the emergence of a new specific type of soils – urbanozem, it is characterized by the disruption of the natural arrangement of horizons, soil regimes, physico-chemical properties, biological activity, etc. [1]. A large concentration of industrial enterprises, motor vehicle emissions, the amount of litter in the territory of the city leads to penetration into the soil a huge number of pollutants, including heavy metals (HM).

One of the most sensitive impact of heavy metals on the soil cover is its biological activity, which reflects such indicators as the functional activity of microorganisms, enzyme activity, respiration rate, etc.

Soil enzymes react with a change of their activity even to minor anthropogenic impact [2] and can be used as bioindicators. To develop biodiagnostics scales, it is necessary to conduct studies in this direction on different types of soils.

2. Material and methods

In this regard, in a model laboratory experiment, the effect of copper and cadmium on the activity of soil enzymes and respiration intensity of urbanozem was studied. HM were introduced into the soil as water solution of sulphate of copper in doses below and above the values of roughly allowable concentration (RAC) (66 mg per kg) - dose 1 is 16.5, dose 2 is 33, dose 3 is 66 and dose 4 is 132 mg per kg of soil and cadmium acetate with RAC of 2 mg per kg – dose 1 is 5, dose 2 is 10, dose 3 is 20 and dose 4 is 40 mg per kg of soil. The activity of catalase, polyphenol oxidase and peroxidase was determined according to the methods described by F Kh Khaziev [3], respiration in A Sh Galstyan [4] on the 3-d, 90-th, 180-th and 360-th day from the beginning of the experiment.
3. Results and discussion

It is known that the enzymes play an important role in the formation of soil fertility and are one of its indicators [5]. Along with this, they have a high sensitivity to such negative environmental factors as heavy metals [6]. The group of redox enzymes include catalase, dehydrogenase, peroxidase, oxidase, etc., which are involved in the processes of transformation of organic matter in soil. Evaluation of the effect of different doses of cadmium and copper on catalase activity showed that doses of contaminants below the values of RAC have an inhibitory effect, which clearly reflects figure 1. This is the evidence of the high toxicity of the studied metals for the enzyme catalase, inhibition of which enhances the negative effect on living organisms because of violation of processes of decomposition of hydrogen peroxide carried out by this enzyme.

Comparative analysis of the influence of metals on catalase activity showed the greater toxicity of cadmium compared to copper on the 3-d, 90-th and 180-th day. Moreover, with increasing dose the inhibitory effect was increased: the percentage of reduction of catalase activity amounted: on the 3-d day after contamination of soil with cadmium to 24-41%, copper - to 12-35%; on the 90-th day – to 10-45% and 5-35%; on the 180-th day – to 13-43% and 12-30%, respectively. However, at the end of the experiment, it was revealed that, the intensification of the processes of decomposition of hydrogen peroxide amounted 11-41% with cadmium contamination, and 11-63% with copper, which may be due to the reduction of their toxicity in connection with transformation of heavy metals in soil and transfer to an inactive status.

![Figure 1](image_url)

**Figure 1.** The percentage change of catalase activity in an urban soil contaminated with different doses of cadmium and copper.

An important role in the processes of humification belongs to the oxidase and peroxidase [3]. In contrast to catalase the activity of peroxidase increased with increasing doses of cadmium and copper in the soil during the first three observation times. On the 3-d and the 90-th day cadmium intensified the oxidation of organic substances, that took place with the participation of this enzyme, to a greater extent than copper: the growth of activity after introducing of cadmium during these periods amounted...
to 6-28% and to 10-55%, in the experiment with copper this figure was equal to 2-23% and 1-6%, respectively. As can be seen from figure 2 on the 180-th day the picture has changed and in variants of experience with copper at doses numbered 1-3 the growth of activity of peroxidase was higher than in the experiment with cadmium and made up 26-31% and 19-29%, respectively. At the end of the experiment, the enzyme activity in the experimental variants with the metals were below control values: copper at doses numbered 1-3 was more toxic for the enzyme (decrease of activity at 6-25%) compared to cadmium (5-14%) (figure 2).

![Activity comparison between cadmium (Cd) and copper (Cu) at different doses](image)

**Figure 2.** The percentage changes in the activity of peroxidase in an urban soil contaminated with different doses of cadmium and copper.

A comparative analysis of the charts in figure 1 and 2 revealed an antagonistic change in the activity of catalase and peroxidase. Similar results were obtained on other soil types [7] contaminated with cadmium, copper, zinc and lead. One of the possible causes of such an addiction, apparently, is participation of hydrogen peroxide in the reactions with these enzymes [3].

The studied metals suppressed the activity of oxidation processes of organic substances with the participation of polyphenol oxidase during the whole experience (figure 3). On the 3-d, 90-th and 360-th day cadmium in a number of variants of the experience was more toxic than copper. On the 180-th day an inverse relationship was observed. In comparison to control value a valid decrease of enzyme activity with cadmium and copper pollution, was installed in all variants of experience and was for cadmium 6-23% on the 3rd day, for copper respectively 6-21%, on the 90-th day it was 5-29% and 1-23%, on the 180-th day – 11-23% and 10-25%, and on the 360-th day – 1-30% and 3-19%.
Figure 3. The percentage changes in the activity of polyphenol oxidase in an urban soil contaminated with different doses of cadmium and copper.

Along with the activity of enzymes heavy metals suppressed the respiration of the soil as well. The increase in the dose of cadmium in urbanozem reduced this figure to 4-52% on the 90-th day, and to 11-76% on the 180-th day, to 2-27% on the 360-th day (figure 4). The introduction of copper in doses of 16.5 and 33 mg per kg increased respiratory rate, respectively to 11-22% by the 90-th day and by the 180-th day within doses of 16.5-66 mg per kg to 6-16% in comparison with the control value. Perhaps, this effect of copper is due to the fact that it belongs to the group of trace elements and is involved in biological processes, intensifying them. Higher copper doses of 66 and 132 mg per kg in soil showed toxic effect in comparison with the control value. Directly proportional dependence of the intensity of respiration and depending on the dose of metal was evident on the 180-th day.

The ambiguous relationship between the dose of metal and respiration, probably, stems from the fact that this indicator is integral and reflects the functional activity of soil biota.

Thus, the decrease in the activity of studied enzymes during the introduction of cadmium and copper on the background of increase of the respiration rate, indicates a high sensitivity of enzymes (molecular level). Affords you the opportunity to recommend this indicator for the diagnosis of early changes in biochemical transformations of organic matter in the soil when small doses of pollution occur. The respiration in these purposes can only be used when cadmium pollutes the soil.
Figure 4. The percentage changes of the respiration rate in an urban soil contaminated with different doses of cadmium and copper.

The obtained dependences of the change in the activity of catalase, peroxidase and polyphenol oxidase can be used to compile an indicative biodiagnostics scales for urbanozem contamination of cadmium and copper after confirmation of the results in field studies.

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