Assessment Of Ecotoxicity Of Thallium By Biological Indicator Of Soil Condition

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Abstract

Thallium is a rare and highly toxic heavy metal. The scale and degree of soil contamination with thallium are increasing every year, and the associated environmental risks have been insufficiently investigated. Model laboratory experiments on the ordinary chernozem pollution with thallium oxide (III) have been carried out. It has been found that the ordinary chernozem pollution with thallium worsened its biological activity. The total bacterial count, the abundance of the Azotobacter bacteria, the activity of catalase, the activity of dehydrogenases, and the germination of radish seeds decreased. The degree of deterioration of the chernozem biological activity depends on the thallium concentrations in soil and the period from the moment of contamination. In the vast majority of cases there is a direct correlation between the thallium content in the soil and the level of soil biological activity reduction. The greatest negative impact of thallium pollution on chernozem biological activity has manifested itself 30 days after contamination. On the 90th day, there was a pronounced tendency to restore the chernozem biological activity. However, the chernozem biological activity did not restore to the initial uncontaminated soil values. At contamination of chernozem with thallium, microbiological indices (the bacterial count and the abundance of the Azotobacter bacteria) were more sensitive, and the enzyme activity indicators (catalase, dehydrogenase) and phytotoxicity (germination of plant seeds) were more informative. All used indicators of biological activity demonstrated high sensitivity to soil contamination with thallium and high correlation coefficients with the thallium content in the soil. Therefore, these biological indicators should be used for monitoring, diagnosis, indication and regulation of soil contamination with thallium.

Introduction

The study of the influence of heavy metal contamination on soil properties has been the subject of numerous studies. However, not all heavy metals are equally well studied. Thallium is one of the least known pollutants. It is present in soil in very small amounts - less than 1 ppm (mg/kg) (Fergusson, 1990; Xiao et al., 2012; Vodyanitskii, 2013; Nelson, Chen, 2017), but is very toxic (Zitco, 1975; Leonard, Gerber, 1997; Zhang et al., 1997), even more toxic than heavy metals such as mercury, cadmium, lead, copper or zinc (Xiao et al., 2004a, b; Peter, Viraraghavan, 2005). The United States Environmental Protection Agency (EPA) indicates it as a priority pollutant.

The sources of soil contamination with thallium are combustion of coal, oil and oil products, ferrous and nonferrous metallurgy, cement production, and the use of rodenticides (Nriagu, Pacyna, 1988; Kazantzis, 2000, Anton et al., 2013; Karbowska et al., 2014).

The highest concentrations of thallium in soil have been observed near the coal mines, including up to 20,000 ppm or 2% of the soil mass (Baceva et al., 2014), which is 100,000 times more than the average thallium content in soil.

The danger exists of thallium spreading to agricultural crops and further along food chains (Tremel et al., 1997; Xiao et al., 2004a; Pavlickova et al., 2005; 2006; He, 2008).
Therefore, it is urgent to identify patterns, mechanisms and possible effects of thallium on soil condition, establish the limits of soil resistance to contamination, and normalize the thallium content in soils.

The purpose of this work is to evaluate the ecotoxicity of thallium by biological indicators of soil conditions.

**Materials And Methods**

Black chernozem was used as an object of the study. These soils are considered to be the most fertile ones in the world. The soil for model studies was selected from the virgin soil top layer (0–10 cm) on the territory of the Persianovskaya Steppe nature monument (Russia, Rostov Region, Persianovskiy, 47°30’33.23”N, 40°9’18.21”E).

The investigated chernozem was characterized by high humus content in the upper horizon – 4.0%, neutral reaction of the medium - pH 7.6, heavy-loamy granulometric composition, good condition, high biological activity: total bacterial count – 3.5 bln/g of soil, catalase activity – 8.4 ml O2/g of soil for 1 min, dehydrogenase activity – 14.5 mg SSF/10 g of soil for 24 hours, an abundance of *Azotobacter* bacteria – 100% of the fouling lumps.

Pollution of chernozem with thallium was simulated in laboratory conditions. Thallium was introduced into the soil in the form of thallium oxide (III) — Tl2O3. Tl (III) was used since it was more toxic than Tl (I) (Ralph, Twiss, 2002; Lan, Lin, 2005). The use of oxides eliminates the influence of the accompanying anions on soil properties, as it occurs at introducing metal salts.

Thallium oxide (III) is a water insoluble compound. To uniformly distribute thallium in the soil, thallium oxide was first ground with a small volume of soil and then mixed with the rest of the soil. After that, the soil was moistened with water.

The content of thallium in the soil is usually up to 1 ppm (from 0.01 to 3.0 ppm) (Fergusson, 1990; Hofer et al., 1990; Chen et al., 1991; Tremel et al., 1997; Il’in, Konarbaeva, 2000; Salminen, 2005; Martínez-Sanchez et al., 2009; Nygard et al., 2012; Stafilov et al., 2013; Alekseenko, Alekseenko, 2013). However, there can also be higher content of thallium in the soil: 5–15 ppm in the pyrite deposit areas (Yang et al., 2005), 40–124 ppm in the thallium sulfide deposits areas (Xiao et al., 2004b), and up to 20,000 ppm near coal mines (Baceva et al., 2014).

The content of thallium in chernozem, used in the study, before pollution is 0,98 ppm (the thallium content in the soil was determined by inductively coupled plasma mass spectrometry using an ELAN-DRC-e instrument). Accordingly, NAC was set to 3 ppm. Thallium was introduced into the soil in the amount of 3, 30 and 300 ppm. It was assumed that the metal toxicity was manifested with its concentrations in the soil from 3 backgrounds (Kolesnikov et al., 2010).
Soil (1 kg) was incubated in plastic vessels in triplicates at room temperature (20–22 °C) and optimal moistening (60% of water field capacity).

The biological activity of the soil was studied since it reacted first to external influences. It was much more sensitive and informative than other chemical and, especially, physical soil properties (Kolesnikov et al., 2000).

The biological activity of chernozem was determined 10, 30 and 90 days after the contamination.

After this period, the entire mass of the soil was removed from the vegetation vessel and mixed, thereby obtaining an “average sample” from which samples were taken to determine biological indices − 3 samples from each vessel.

Laboratory-analytical studies were performed using conventional methods. The total bacterial count, the abundance of the *Azotobacter* bacteria, the activity of catalase and dehydrogenases, and phytotoxic properties of soil were determined. The total bacterial count in the soil was taken into account using luminescent microscopy (n = 720: 3 incubation vessels with soil x 3 soil samples x 4 square centimetres on slides x 20 fields of vision), *Azotobacter* - by the lumps fouling in the Ashby medium (n = 241: 3 incubation vessels with soil x 3 soil samples in Petri dishes x 25 fouling lumps), the catalase activity was determined by the decomposition rate of hydrogen peroxide (n = 36: 3 incubation vessels with soil x 3 soil samples x 4 analytical replicates), dehydrogenase activity - by the rate of conversion of triphenyltetrazolium chloride into triphenylformazan (n = 36: 3 incubation vessels with soil x 3 soil samples x 4 analytical replicates), and soil phytotoxicity was judged by the germination of radish seeds (n = 241: 3 incubation vessels with soil x 3 soil samples in Petri dishes x 25 radish seeds).

The choice of biological indicators is due to the following reasons. The total bacterial count in the soil characterizes the state of the decomposers in the ecosystem. The *Azotobacter* bacteria are traditionally used as an indicator of chemical soil contamination. The activity of catalase and dehydrogenases reflects the intensity of mineralization processes in the soil. Among enzymes, oxidoreductases are the most sensitive to chemical contamination. Enzyme activity is an indicator of the potential biological activity of the soil, and the decomposition rate of the bed characterizes the actual biological activity of the soil.

Based on the above biological indicators, the integral biological state indicator (IBSI) of the soil was determined (Kolesnikov et al., 2000). The presented set of indicators gives an informative picture of the biological processes taking place in the soil and its ecological state.

To calculate IBSI, the value of each of the above indicators for control (in the unpolluted soil) was taken as 100%, and the values in the remaining experiment options were expressed in relation to it as a percentage (in the contaminated soil). Then, the average value of the five selected indicators for each experiment option was determined. The value obtained (IBSI) was expressed as a percentage of the
control (to 100%). The technique used allowed integrating the relative values of different indicators, the absolute values of which could not be integrated since they had different units of measurement.

To verify the reliability of the data obtained, the variance analysis was carried out, followed by the determination of the least significant difference (LSD).

**Results**

It has been established that the pollution of ordinary chernozem with thallium in all variants of the experiment leads to deterioration of biological activity (Table 1). The total bacterial count, the abundance of the *Azotobacter* bacteria, the activity of catalase, the activity of dehydrogenases, and the germination of radish seeds decrease.
| Exposition period, days | Element content in soil, % | Control | 1 UDC (3 ppm) | 10 UDC (30 ppm) | 100 UDC (300 ppm) | LSD_{0.05} |
|------------------------|--------------------------|---------|---------------|-----------------|-------------------|------------|
|                        |                          |         |               |                 |                   |            |
| Total bacterial count  |                          |         |               |                 |                   |            |
| 10                     |                          | 100     | 54            | 39              | 24                | 7          |
| 30                     |                          | 100     | 60            | 37              | 24                | 7          |
| 90                     |                          | 100     | 61            | 44              | 32                | 7          |
| LSD_{0.05}             |                          | 3       | 3             | 2               |                   |            |
| Abundance of Azotobacter bacteria |         |         |               |                 |                   |            |
| 10                     |                          | 100     | 84            | 73              | 60                | 10         |
| 30                     |                          | 100     | 82            | 79              | 62                | 10         |
| 90                     |                          | 100     | 81            | 74              | 64                | 10         |
| LSD 0.05               |                          | 5       | 5             | 5               |                   |            |
| Catalase activity      |                          |         |               |                 |                   |            |
| 10                     |                          | 100     | 98            | 81              | 35                | 9          |
| 30                     |                          | 100     | 98            | 88              | 39                | 10         |
| 90                     |                          | 100     | 94            | 84              | 38                | 10         |
| LSD 0.05               |                          | 5       | 5             | 5               |                   |            |
| Dehydrogenase activity |                          |         |               |                 |                   |            |
| 10                     |                          | 100     | 97            | 85              | 82                | 11         |
| 30                     |                          | 100     | 97            | 96              | 92                | 12         |
| 90                     |                          | 100     | 98            | 94              | 78                | 11         |
| LSD 0.05               |                          | 5       | 6             | 6               |                   |            |
| Germination of radish seeds |                |         |               |                 |                   |            |
| 10                     |                          | 100     | 92            | 95              | 83                | 10         |
| 30                     |                          | 100     | 95            | 91              | 81                | 11         |
| 90                     |                          | 100     | 99            | 98              | 96                | 12         |
| LSD 0.05               |                          | 5       | 6             | 6               |                   |            |
Integral biological state indicator of the soil (IBSI),

| Sensitivity | Informativity |
|-------------|---------------|
| 10          | 100           |
| 30          | 100           |
| 90          | 100           |

The degree of reduction depends on the thallium concentrations in soil and the period from the moment of contamination. In most cases, a direct relationship between the thallium concentration and the degree of deterioration in the chernozem biological activity has been noted. An assessment of the chernozem biological activity dynamics showed that the greatest negative impact of thallium pollution on chernozem biological activity had manifested itself 30 days after the contamination. On the 90th day, there was a pronounced tendency to restore the biological activity of the chernozem. However, the biological activity of the chernozem was not restored to the initial uncontaminated soil values.

One of the study objectives was to assess the degree of informativity and sensitivity of various biological indicators and to determine the appropriateness of use thereof to monitor, diagnose and regulate pollution of soils and ecosystems as a whole with thallium.

The sensitivity of the indicator was assessed by the degree of decrease in its values in the options with contamination compared to the control (Table 2). The informative value of the indicator was estimated from the tightness of the correlation between the index and the thallium content in the soil - the correlation coefficient (Table 2).

| Indicator                             | Sensitivity | Informativity |
|---------------------------------------|-------------|---------------|
| Bacterial count                       | 57          | -0.70         |
| Abundance of *Azotobacter* bacteria   | 59          | -0.83         |
| Catalase activity                     | 88          | -0.90         |
| Dehydrogenase activity                | 79          | -0.88         |
| Germination of radish seeds           | 93          | -0.94         |
| IIBS                                  | 75          | -0.87         |

1 The Indicator Sensitivity is the degree of decrease in the biological index at soil contamination with thallium, % of the control (the values are averaged over doses and the terms of contamination);

2 Informativity of the indicator is the correlation ratio ($r$) between the thallium content in the soil and the biological index ($\alpha=0.05$).
Discussion

Apparently, the reasons for the negative impact of thallium on the biological activity of soils are the same as those of other heavy metals — reduced permeability of biological membranes, inhibition of enzymes, and metabolic disorder as a result. The mechanisms of thallium toxicity are the violation of the potassium transport through the mitochondrial membrane and the Na/K pump (Repetto et al., 1998), as well as the binding of thallium to N and S ligands, stronger than that of potassium (Williams, Frausto da Silva, 1996). The similarity of the biochemical and geochemical behavior of thallium and potassium is explained by the approximate sizes of their atomic radii.

In some cases, at soil contamination with the lowest of the investigated doses of thallium (1 UDC), a statistically unreliable increase in the biological activity values was observed. In ecotoxicology, there exist cases of stimulating action of various chemicals that enter living organisms or soil in small quantities (Zvyagintsev et al., 1997; Kolesnikov et al., 1999; Kabata-Pendias, 2010). They were called the "small doses effect" (Southam, Ehrlich, 1943).

By the degree of sensitivity (Table 2) to soil contamination with thallium, biological indicators form the following series: total bacterial count $\geq$ abundance of the $Azotobacter$ bacteria $\geq$ dehydrogenases activity $>\text{catalase activity}>\text{radish germination}$.

By the degree of informativity (Table 2), biological indicators form the following sequence: radish germination $>\text{catalase activity}>\text{dehydrogenases activity}>\text{abundance of } Azotobacter \text{bacteria}>\text{total bacterial count}$.

Consequently, at contamination of chernozem with thallium, microbiological indices (the bacterial count and the abundance of the $Azotobacter$ bacteria) were more sensitive, and the enzyme activity indicators (catalase, dehydrogenase) and phytotoxicity (germination of plant seeds) were more informative. Similar patterns are characteristic for most heavy metals and metalloids (Kolesnikov et al., 2000, 2008, 2009).

Conclusion

The ordinary chernozem pollution with thallium has worsened its biological activity. The total bacterial count, the abundance of the $Azotobacter$ bacteria, the activity of catalase, the activity of dehydrogenases, and the germination of radish seeds decreased.

The degree of deterioration of the chernozem biological activity depends on the thallium concentrations in soil and the period from the moment of the contamination. In the vast majority of cases, there is a direct correlation between the thallium content in soil and the level of soil biological activity reduction.
The greatest negative impact of thallium pollution on chernozem biological activity manifested itself 30 days after contamination. On the 90th day there was a pronounced tendency to restore the biological activity of the chernozem. However, the biological activity of the chernozem was not restored to the initial uncontaminated soil values.

At contamination of chernozem with thallium, microbiological indices (the bacterial count and the abundance of the *Azotobacter* bacteria) were more sensitive, and the enzyme activity indicators (catalase, dehydrogenase) and phytotoxicity (germination of plant seeds) were more informative.

All used indicators of biological activity have demonstrated high sensitivity to soil contamination with thallium and high correlation coefficients with the thallium content in the soil. Therefore, these biological indicators should be used for monitoring, diagnosis, indication and regulation of soil contamination with thallium.

**Declarations**

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