UDC: 631.41:622.34
review paper

Acta Agriculturae Serbica, Vol. XI, 22 (2006) 45-55

Acta Agriculturae Serbica

Microorganisms as Indicators of Soil Pollution with Heavy Metals

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Abstract: Since indication geobotany and indication biology have not succeeded to identify microorganisms for early indication of soil pollution yet, microbiological indication has been approached differently - analysing diagnostic signs of isolated levels of soil pollution with heavy metals. In this regard, the distribution occurring in the amylolytic microbial community under the effect of the anthropogenic soil loading and particular indicators of biochemical activity of soil microorganisms (nitrogen fixing activities) should be analysed when studying responses of higher plants and the occurrence of microorganisms, which are resistant to soil pollution with heavy metals.

Key words: soil, pollution, heavy metals, microorganisms.

Introduction

Environmental pollution with heavy metals is a global issue. It is present everywhere, though to different degrees, and is specific to certain parts of the biogeosphere (Djukic, 1992; Djukic et al., 1996; Kastori, 1997). The environment is polluted by numerous organic and inorganic compounds, heavy metals in particular. Living organisms are not able to prepare and adapt rapidly to a sudden and huge environmental load with different toxic substances, and therefore, the accumulation of certain elements, especially of heavy metals with mainly toxic effect, can cause undesirable changes in the biosphere with unforeseeable consequences (Djukic and Mandic, 2000a).

With this and the pronounced cumulative soil capacity for different kinds of xenobiotics in mind, we have considered it very appropriate to examine the possibility of microbiological indication of soil pollution with heavy metals.

Microbiological indication of soil pollution with heavy metals

An important activity in environmental preservation is searching for and establishing necessary criteria and manners of soil pollution indication. Efforts of microbiological indications of soil pollution are accompanied by serious difficulties. Traditional soil microbiology methods cannot provide successful early indication. On the one hand, the difficulty is a masking effect of dormant microorganisms, which survive in the soil for a long time, and on the other – considerable fluctuations in the number and biochemical activity of specific systematic groups of microorganisms. Numerous efforts to detect indicator microorganisms have not resulted in a common solution to the problem. Without disputing the cause-and-effect links between pollutant effects and changes in the microbial soil system (Djukic, 1992; Djukic et al., 1996), it should be borne in mind that alterations at low and medium pollution levels will refer to different groups of microorganisms (graphs 1, 2, 3, 4). Moreover, pollutants differing in the effect they produce can cause analogous changes. This makes the interpretation of the results obtained very difficult and detection of indicator microorganisms for early indication of soil pollution pointless. It should be emphasised that a borderline discipline – indication geobotany, also, has not had great success in this sense. Indication biology as well is still at the ”preindication” development stage (Mjalo, Gorjainova, 1980; Mirkin, 1985).

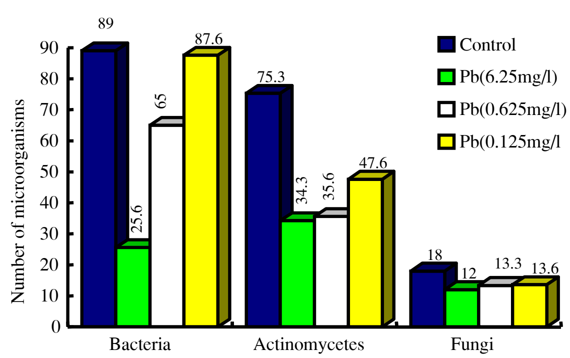

Fig. 1. Effect of diverse Pb concentrations on the total number of bacteria (10^6/1g absolutely dry soil), number of fungi and actinomycetes (10^5/1g absolutely dry soil) – *Djukc et al., 1996.*

Isolated levels of soil pollution with heavy metals and their diagnostic signs enable a different approach to the microbiological indication issue.

The provisionality of the boundary between a non-contaminated soil and soil with low pollution level, as well as a significant variability in micro-biological indicators prevent performance of microbiological indication of low pollution level. In that case chemical analysis should be adequate for the purpose since it is incomparably simpler to carry out and the concentration of heavy metals in the studied soil can be determined with certainty. It should be borne in mind here that nominal values of basic concentrations (which are unique for all soils) are relative (Zirin et al., 1985), due to which basic concentrations of heavy metals characteristic of concrete soil-geochemical entity (space) should be considered.

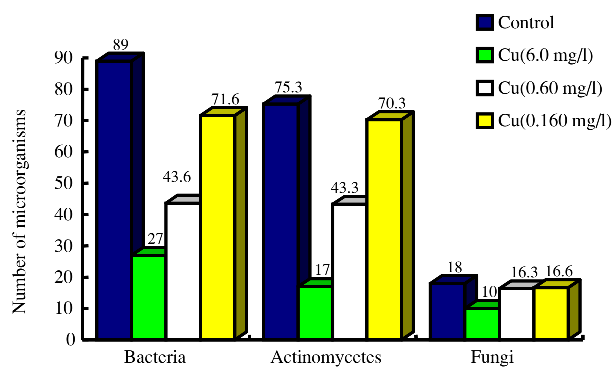

Fig. 2. Effect of diverse Cu concentrations on the total number of bacteria (10^6/1g absolutely dry soil), number of fungi and actinomycetes (10^5/1g absolutely dry soil) - *Djukic et al., 1996.*

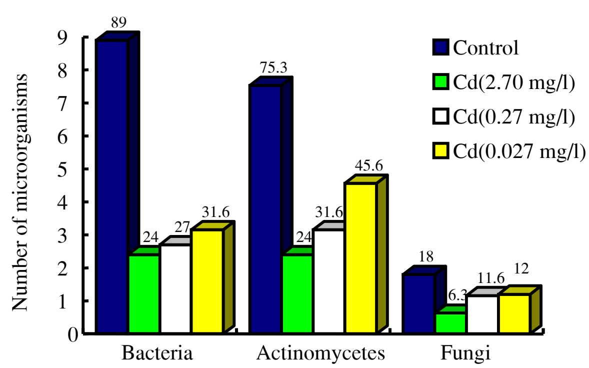

Fig. 3. Effect of diverse Cd concentrations on the total number of bacteria (10^6/1g absolutely dry soil), number of fungi and actinomycetes (10^5/1g absolutely dry soil) - *Djukic et al., 1996.*

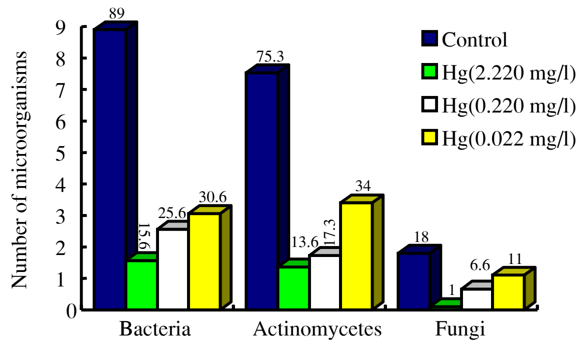

Fig. 4. Effect of diverseHg concentrations on the total number of bacteria (10^6/1g absolutely dry soil), number of fungi and actinomycetes (10^5/1g absolutely dry soil) - *Djukic et al., 1996.*

When the concentration of heavy metals in the soil is above their basic level it is possible to make a distinction between non-contaminated soils and the ones with a low pollution level. However, using this trait, it is not possible to determine the boundary between the low pollution level and the next, medium level. Microbiological indicators are the most appropriate for indication of this pollution level because at a medium pollution level there occur changes in the composition of the soil microbial community which have adverse effects (Djukic and Mandic, 2000b; Mandic et al., 2002). As seen from the analysis of diagnostic traits of this level, the occurrence of negative properties in the soil is directly associated with the distribution of activities of special components in the complex of soil microorganisms. For example, the proportion of epiphytic chromogenic forms of microorganisms and the amount of toxinogenic micromycetes are increased in the soil. Owing to this, the redistribution of active microorganisms according to the domination degree can be justifiably considered as a basic property of pre-pathological stage of soil microbial community. In this respect, and for the purpose of indication, a factor of redistribution in any group of microorganisms should be determined, with the redistribution being directly linked to the polluter effect. However, there are still no commonly accepted methods which should provide direct determination of the fact. Direct methods use too broad taxonomic categories, and the masking effect of dead forms of microorganisms hinders the application of the method of introduction into nutritive media. Evidently, a complex of indicators, including every possible change in the soil microbial community, cannot be used for indication, but a simple test is required which would clearly (synonymously) point to the link between the anthropogenic load and microbiological reaction. In our opinion, the simplest test is the response of initiated amylolytic microbial community to soil pollution. Redistribution in the amylolytic microbial community under the effect of the anthropogenic load can be easily identified in laboratory conditions. Changes in the composition of other groups of soil microorganisms are likely to happen in the similar way, as confirmed by the example of cellulolytic initiated microbial community (Kurakov, 1983; Djukic and Mandic, 1997). This can be used to propose a manner of indication of soil pollution with heavy metals, which is based on the introduction of pollutants into the soil at the rate identical to the doubled concentration characteristic of the homeostasis zone.

The introduction of this rate into the non-contaminated soil causes the redistribution according to the degree of domination of microorganisms of initiated community. If this should happen, it would mean that the studied soil is unpolluted or that it has a low pollution level. If the soil is characterised by medium pollution level, additional introduction of the mentioned rate does not necessarily lead to described changes, because they have already taken place in field conditions. The proposed manner of the microbiological indication of soil pollution is rather simple to perform and suitable for wider use. First, it does not necessarily need control samples, because there is no comparison between absolute but rather between relative differences in the feedback of microbial community to the additional introduction of the pollutant. Samples of unpolluted soil of equal type can be used as the control. Secondly, the performance of such indication does not require a too experienced microorganism systematist. In microbial community dominant forms with clear morphological properties should be differentiated, but their species identification is not needed. In other words, for certain purposes, morphological types, which will be most likely presented to different species can be used. As a way of illustration, the results of the vegetation trial with the introduction into the soil of mercury compounds with different solubility degrees can be used. During additional introduction of mercury (at the rate of 4 mg/kg soil) in the form of nitric acid salts (the size of the homeostasis zone for the compound is 2 mg/kg) into the soil previously polluted with the same mercury compound, the redistribution of dominant microorganisms of the initiated microbial community occurs only in the control variant of the soil (graph 5). In all other variants, during additional introduction of pollutant, the redistribution does not occur, because it occurred earlier. Based on this, the variants mentioned are classified as medium pollution level. In an analogous experiment (graph 6), where at the beginning, a poorly soluble mercury sulfide (the size of the homeostasis zone for the compound is 10 mg/kg) is introduced into the soil, followed by the mercury salt of nitric acid (at the rate of 4 mg/kg), the redistribution occurs in the control and in the variant where mercury is introduced at the rate of 10 mg/kg (in the form of sulfide). That variant can be classified as a variant with low pollution level, and the other ones – as medium pollution level.

The manner proposed, including organisation, the composition of initiated microbial community and redistribution of community members, according to the domination degree, can be accepted as a basis for indication of medium level of soil pollution with heavy metals. Evidently, as an additional indication trait, changes in special indicators of biochemical activity of soil microorganisms can be used, for example, the nitrogen fixing activity, and particularly the change in its variability. When describing the response of soil microbial community to the medium pollution level, changes with negative aspect should be indicated, such as a reduction in the abundance of species and in the diversity of complexes of soil microorganisms, domination of toxinogenic micromycetes etc. For instance, in the trials already described, where mercury concentration in the soil exceeds 50 mg/kg (which is considerably higher than its basic content in the soil), the composition of the amylolytic microbial community is dominated by toxinogenic microorganisms – *Penicillium furiculosum* (graph 5) and *P. vermiculatum* (graph 5, 6). Their high phytotoxic activity is determined as follows. The seed of the test plant – garden cress (*Lepidium sativum*) is spread over the surface of the soil plate, where the microorganisms mentioned have developed. The soil with identical mercury concentrations is used as a control, but initiating substrate (starch) is not introduced and therefore active development of microorganisms does not ensue. Therefore the seed spread over the surface of the starch plate covered by mycelium does not germinate, whereas 50-80% of seed spread on the soil without microbial growth does. In this case, a medium level of soil pollution with mercury with the domination of toxinogenic microorganisms can be recorded.

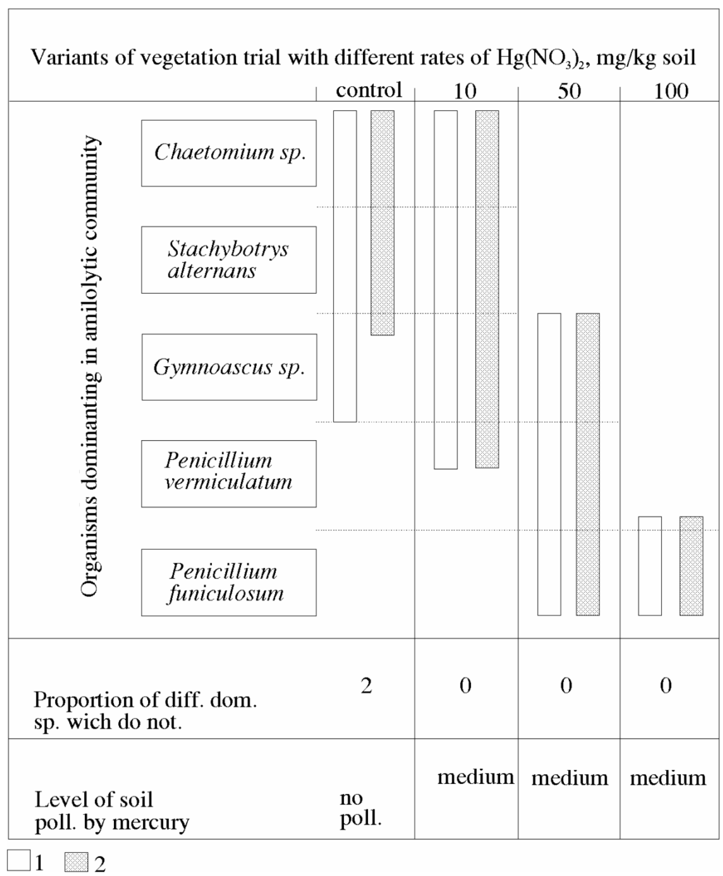

Fig. 5. Change of predominant composition of amylolytic microbial community of podzol soil as a response to additional incorporation of mercury-$Hg(NO_3)_2$ - 4 mg/kg soil: 1-original community; community after mercury application
Soil was previously contaminated by the same mercury compound.

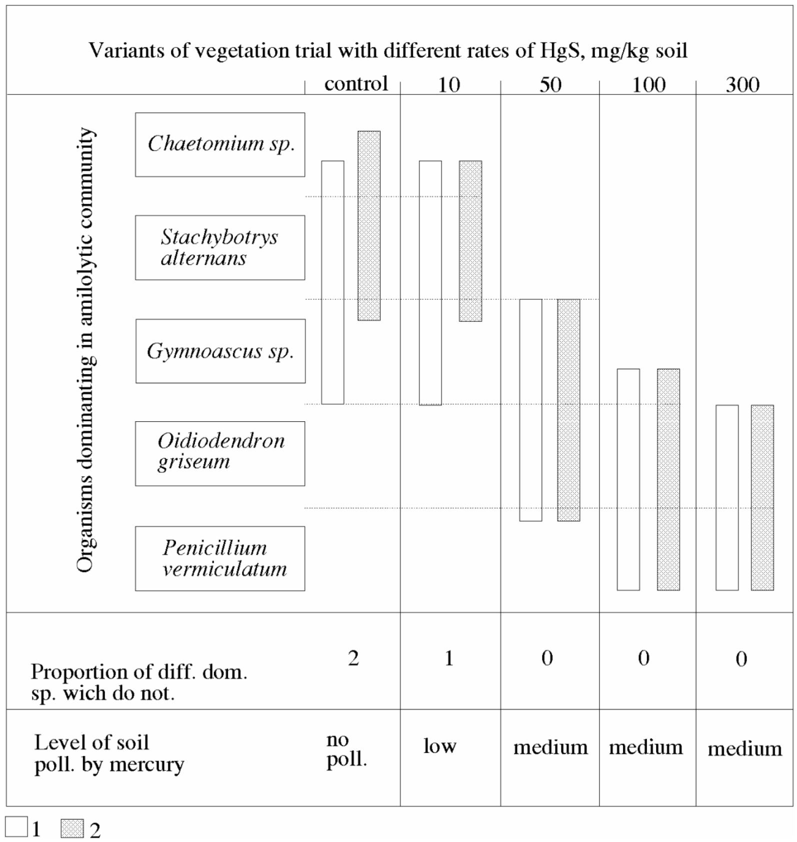

Fig. 6. Change of predominant composition of amylolytic microbial community of podzol soil as a response to additional incorporation of mercury-$Hg(NO_3)_2$ - 4 mg/kg soil: 1-original community; community after mercury application
Soil was previously contaminated by mercury in the form of sulfide (HgS).

Almost all microbiological indicators are suitable for indication of high level of soil pollution with heavy metals, since at the given load level, the majority of them should strongly differ from the analogous indicators, in unpolluted soil of the same type. In our opinion, the most illustrative indicator of the change in soil microbial community in highly polluted soil is the occurrence of resistant forms of microorganisms, which are capable of expressing specific stability toward a pollutant, and moreover, of actively developing in its presence. These very microorganisms are used in geological microbiology (Rozanova, Kuznecov, 1974; Hood et al., 1975). Irrespective of the fact that the high level of soil pollution with heavy metals can be quite easily determined according to microbiological indicators (Djukic et al., 1996, graph 1, 2, 3, 4), we think that other tests are more appropriate. As the soils at the given pollution level are highly toxic, the existence of this level can be more appropriately, and obviously more simply, determined by monitoring of the plant response (Obuhov i sar., 1980; Iljin, 1986), as well as of indicators of heavy metal accumulation in the phytomass etc. (Zirin et al., 1985; Petrovic et al., 1990; Kastori et al., 1993; Petrovic, Kastori, 1994, Kastori et al., 1997. tab. 1, 2).

Tab. 1. Heavy metal content of soil and pasture plants at different distances from lead-zinc smelters (*Kastori et al., 1997, cit. Vetter et al., 1974*)

Elements	**Distance in km**				
	0.75	**1.50**	**3.00**	**6.00**	**12.00**
	kg/ha at 0-80 cm soil depth				
Zn	7500	1850	620	350	240
Pb	2700	730	360	160	130
Cd	71	14	4	1.9	0.8
	mg/kg DW in pasture plants				
Zn	523	118	116	74	78
Pb	98	55	11	12	7
Cd	2.1	1.1	0.6	0.4	0.2

Tab. 2. Cadmium uptake by different crop species grown on a soil enriched with 10 ppm cadmium derived from sewage sludge *(Kastori et al., 1997, cit. Marschner, 1983)*

Crop species	**μg Cd g^{-1} dry matter**	
	Above-ground plant parts	**Seed, fruit, or edible root**
Rice	2.0	-
Sudan grass	6.0	-
White clover	7.0	-
Alfalfa	8.0	-
Bermudagrass	8.9	-
Bean	10.2	1.6
Wheat	11.4	5.8
Squash	12.3	1.8
Tall fescue	14.1	-
Soybean	16.2	9.6
Corn	26.0	1.9
Carrot	36.2	14.6
Cabbage	37.0	2.0
Radish	38.1	3.9
Red beet	44.0	5.0
Lettuce	58.7	-
Tomato	66.2	3.0
Spinach	161.0	-
Turnip	162.0	9.3

Therefore, with the aim of indicating different levels of soil pollution with heavy metals in real field conditions, the following can be proposed. Low pollution level can be detected according to an increase in basic concentrations of heavy metals using common methods of chemical analysis. Medium pollution level can be most clearly established based on the absence of redistribution of members of initiated microbial soil community following the additional introduction of a double pollutant rate compared to the one adequate to the size of the homeostasis zone of unpolluted soil. Additional indication signs may include: a reduction of the nitrogen-fixing activity in the soil and its variability, a decrease in the abundance of species and in the diversity of soil microorganism complexes and an increase in the number of toxinogenic forms, epiphytic and pigmented microorganisms. As regards indication of high pollution level, the most appropriate thing to do is to consider the response of higher plants to pollution. The soil presence of the high population density of microorganisms resistant to a specific pollutant can be used as an additional trait.

Conclusion

An attempt has been made toward analysing the changes in soil microorganism composition due to the anthropogenic factor in order to assess such effect from the aspect of a special scientific discipline - soil microbiology. The criteria and manners proposed for this assessment with an emphasis on the peculiarity of the response of soil microorganisms to heavy metals, have been explained in detail. Different levels of anthropogenic load have been detected and their diagnostic traits have been described. The assessment ways and indicators proposed for microbiological indication of the soil pollution with heavy metals, have also been worked out.

To which extent this approach to the environmental protection from soil pollution with heavy metals will be prospective in other scientific fields, remains to be seen in the future.

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MIKROORGANIZMI KAO INDIKATORI ZAGAĐENOSTI ZEMLJIŠTA TEŠKIM METALIMA

- pregledni rad -

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Rezime

Polazeći od činjenice da u indikacionoj geobotanici i indikacionoj biologiji još uvek nisu postignuti značajniji uspesi u pravcu pronalaženja mikroorganizama za ranu indikaciju zagađenosti zemljišta, smatrali smo neophodnim da se problemom mikrobiološke indikacije priđe na drugačiji način - analizom dijagnostičkih obeležja izdvojenih nivoa zagađenosti zemljišta teškim metalima. U tom smislu treba pribeći, pre svega, analizi preraspodele u amilolitskoj mikrobnoj zajednici do koje dolazi pod uticajem antropogenog opterećenja zemljišta, kao i analizi posebnih pokazatelja biohemijske aktivnosti zemljišnih mikroorganizama (na primer, azotofiksirajuće aktivnosti), praćenju reakcije viših biljaka i pojave mikrootganizama koji su rezistentni na zagađenje teškim metalima.