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CHANGES OF DEHYDROGENASE INHIBITION IN SOIL INDUCED BY VARIOUS CONCENTRATIONS OF SELECTED HEAVY METAL SALTS

Janina Nowak, Bożena Śnieg
Department of Biochemistry, Agricultural University of Szczecin, Poland

ABSTRACT

The influence of various concentrations of salts of lead (II), copper (II), manganese (II), cobalt (II), molybdenum (IV) and iron (III) upon the soil dehydrogenase has been studied. Ecological areas featuring the contamination degree to which the studied soil environment had been exposed have been determined. Particular attention has been paid to high heavy metal doses causing significant disturbance to soil metabolism.

Key words: heavy metals, soil dehydrogenase

INTRODUCTION

Natural enzymatic activity is an informative index of fertility and productivity of soil. It is multi-factor dependent and shaped by the type of soil, intensity of cultivation, plant cover, vegetative period as well as by many other factors [3,6,12,15,16,17,18].The natural metabolism in soils has been significantly disturbed by overusing pesticides, inorganic and organic fertilizers and main communication ways localised in the proximity of farming fields.

Contamination, especially with heavy metal elements, affects detrimentally individual cells, organisms and even entire ecosystems, via which the harmful influence upon enzymatic activity of soil is transferred [1,4,5,12,15].

Chemical elements introduced into the soil via a variety of sources are the reason to degradation of biological properties of soil, pollution of ground waters and intrusion of the contaminating elements into food chains. The grave harmfulness of heavy metal elements originates from their biochemical properties, more particularly from their susceptibility to bioaccumulation [11,14].

As it has been proved by studies performed so far, the increase in lead concentration in the upper horizons of soils affects negatively both its micro-flora and micro-fauna. Limited enzymatic activity of microorganisms present in the soil, represses decomposition processes of the organic matter, which leads directly to further degradation of soil properties. Soils are also exposed to copper pollution originating from numerous sources. Typical entrances for copper to be introduced are mineral and organic fertilizers, protection and prevention preparations for plants as well as communal waste. In many cases copper is a real threat to the soil, its chemical balance and, similarly to other heavy metals, its concentration leads to degradation of the soil environment. The occurrence of manganese in soils depends both on its concentration in the bedrock and pedogenesic which generates the distribution profile. Intensive mineral and natural fertilization of soils can result in the excessive amount of manganese. Cobalt resembles iron in the respect of its geochemical features: it is non toxic and does not cause severe environmental threats. Its concentration in natural solutions of soils is the lowest when compared to other heavy metal elements. Molybdenum, dispersed over the lithosphere, reaches rather low concentrations in soils. Higher concentrations can be found in ferruginous or humus horizons. Iron constitutes one of the major elements of the globe. In soils it can be found in a variety of compounds, both in crystalline and amorphous forms. Iron compounds significantly influence chemical changes of other elements, particularly from the trace elements group [9,10,11].

Activity of enzymes is disturbed almost immediately after metals join the matter circulating in the soil. Though usually the enzymatic activity returns to its initial level with time, for high concentrations of heavy metal elements a solid inhibition resulting in disturbed dynamic balance of soil transformations may occur [16,17].

The main objective of the reported studies was to investigate the influence of various concentrations of heavy metal salts occurring in soil environments, differing in the oxidation state, upon the dehydrogenase inhibition and its potential threats.

SPECIMENS AND METHODOLOGY

The research has been conducted under laboratory conditions on specimens taken from the ontohumus horizon (0-30 cm) of Black Earths at Gummieniecka Plain, classified as II, IIIa and IVb arable lands within the Polish soil taxonomy. The granular composition of the soils is typical for a light silty clay, the humus content varies from 1.2% to 1.8%, they are of slightly acid or neutral reaction and exhibit high potassium and magnesium availability [2].

The soil material was subsequently injected with aqueous solutions of the following salts: $Pb(NO_3)_2$, $Cu(NO_3)_2 \cdot 3H_2O$, $Mn(NO_3)_2 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$ and $Na_2MoO_4 \cdot 2H_2O$ in the amounts presented in Table 1.

Table 1. Doses of heavy metal salts applied to the soil specimens

Salt of	I concentration [0.05 mM·kg^{-1}] [g·kg^{-1}]	II concentration [0.5 mM·kg^{-1}] [g·kg^{-1}]	III concentration [5 mM·kg^{-1}] [g·kg^{-1}]	IV concentration [50 mM·kg^{-1}] [g·kg^{-1}]
lead (II)	0.0165	0.165	1.65	16.5
copper (II)	0.0123	0.123	1.23	12.3
manganese (II)	0.0143	0.143	1.43	14.3
cobalt (II)	0.0145	0.145	1.45	14.5
molybdenum (IV)	0.0251	0.251	2.51	21.5
iron (III)	0.0178	0.178	1.78	17.8

Four doses of metals were applied, namely a basic one of 0.05 mM per kg of soil, and further three: a ten times higher but still within the limit of Polish Standards, a hundred times higher, and finally a thousand times higher one. Once the aqueous solutions of heavy metal salts have been introduced, moisture in the soil have been balanced at 60% m.p.w. Next, 1 kg specimens were carefully mixed and stored in tight containers that were kept at the temperature of 20°C. The 'control specimen', with no heavy metal salts added, served as a reference sample.

Dehydrogenase activity in for all the studied specimens was measured in four repetition cycles in the 1st, 3rd, 7th, 14th, 28th, 56th and 112th day proceeding the start of the experiment. A spectrophotometric modified Malkomes method [13] and Perkin Elmer UV/VIS (Lambda Bio) spectrometer with the wavelength of 540 nm, and TTC (chloride 2,3,5-triphenylotetrazole) used as a substrate were applied to measure the activity of the studied enzyme.

The results are presented in figures 1 to 6. The data on dehydrogenase activity were read as µg TPF per 1g of dry mass soil and 2 hour incubation time [µg TPF · g^{-1} s.m. $2h^{-1}$], on the base of which the data was then recalculated as the percentage related to the control soil. The activity of the control soil specimen has been set as 100%, hence the values higher and lower than 100% meant activation or inhibition of the enzyme, respectively. In the next step the data have been recalculated once more with relation to the percentage of inhibition of the enzyme taking the inhibition in the control soil sample as 0%. Such a presentation of the results - in most cases - helped to emphasise the clearly inhibiting character of the heavy metal salts influence.

In our studies we also adopted the pattern of plots proposed by Domsch et al. [8] illustrating the areas under ecological threat. Three types ecological areas have been determined. In the first area the found values were negligible, i.e. such that minimally deactivated the concerned enzyme. In the second, the biggest in size, was called 'tolerable', since though the magnitude of the enzyme activity was significantly influenced, the heavy metal concentrations did not cause the soil environment to degrade. In the last 'critical' area, the range of concentration was found to influence the environment most detrimentally and could possibly destroy the microorganisms present in the soil. Comparing the data on dehydrogenase inhibition to particular concentrations of the applied heavy metal salts, it was possible to find which of the doses applied in the experiment significantly disturbed the metabolism in the studied soils.

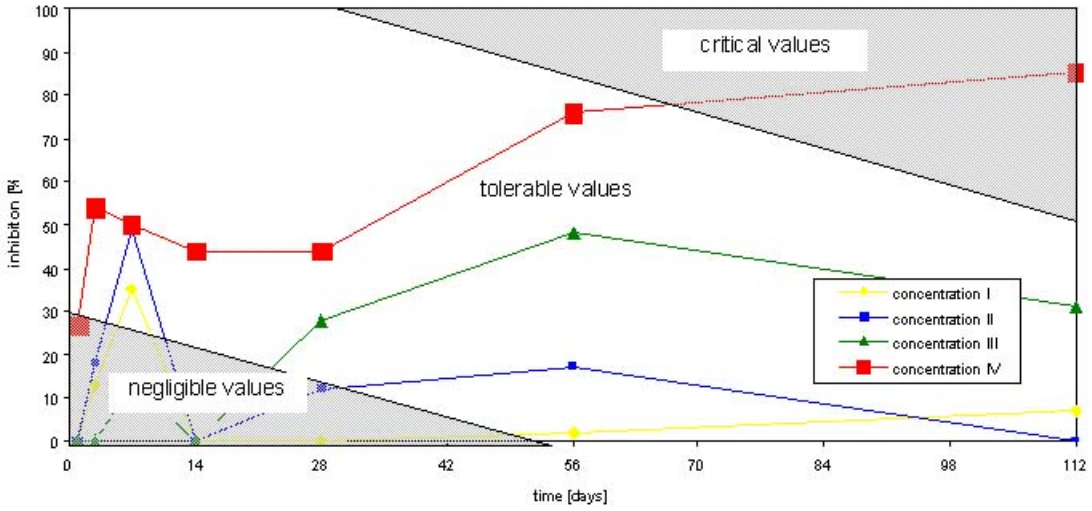
PRESENTATION OF THE RESULTS

The pH of the soil samples to which solutions of heavy metal salts had been added, has been determined and is presented in Table 2. Apart from molybdenum VI salt, concentration IV (50 mM·kg^{-1}) for all the salts significantly changed the degree of acidity in the soil.

Table 2. The pH values for the control and other soil specimens after applying the solutions of heavy metal salts

Metal	Control		Concentration II		Concentration II		concentration III		Concentration IV	
	pH H_2O	pH KCl	pH H_2O	pH KCl	pH H_2O	pH KCl	pH H_2O	pH KCl	pH H_2O	pH KCl
Pb (II)	7.74	7.52	7.72	7.50	7.73	7.55	7.75	7.31	5.41	6.39
Cu (II)	7.88	7.41	7.68	7.39	7.72	7.47	7.28	7.33	5.75	6.07
Mn (II)	7.88	7.41	7.69	7.36	7.39	7.27	7.27	7.20	6.07	6.60
Co (II)	7.48	6.89	7.67	7.33	7.66	7.31	7.32	7.16	6.37	6.30
Mo (VI)	7.55	7.12	7.59	7.16	7.52	7.17	7.94	7.21	8.34	8.15
Fe (III)	7.48	6.89	7.57	7.03	7.48	7.06	7.19	7.00	4.97	4.89

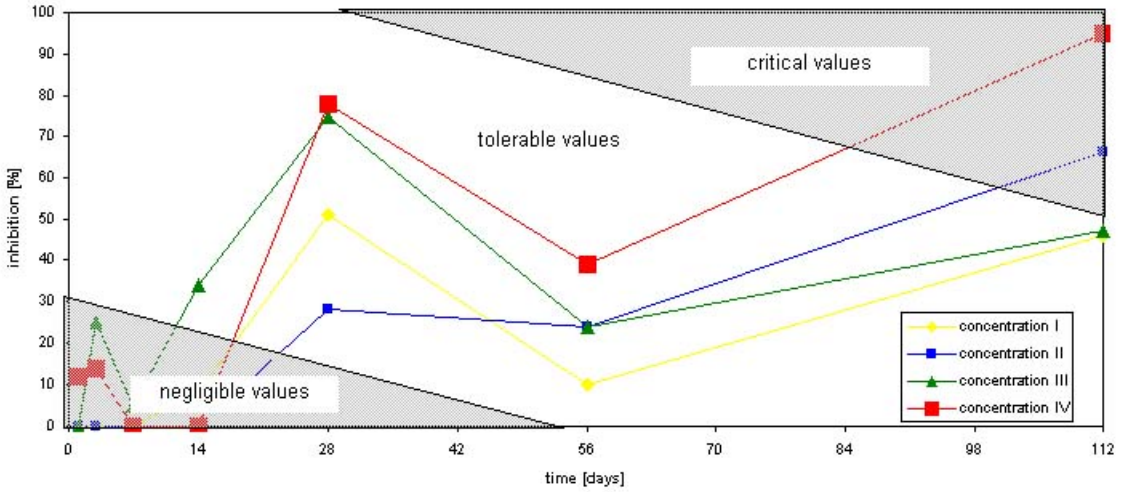
Fig 1. The degree of dehydrogenase inhibition caused by various concentrations of lead (II) salt related to the ecological significance of soil environment pollution



The degree of dehydrogenase inhibition caused by various concentrations of lead (II) salts applied to the studied soil specimens is presented in Fig.1. For concentration I [0.05 mM·kg^{-1}] inhibition was found insignificant and its values, apart from day 7 of the measured series, fell within the range of 'negligible magnitudes' until the experiment was half completed. Since 56^{th} day till the end of the experiment the obtained data fell into the range

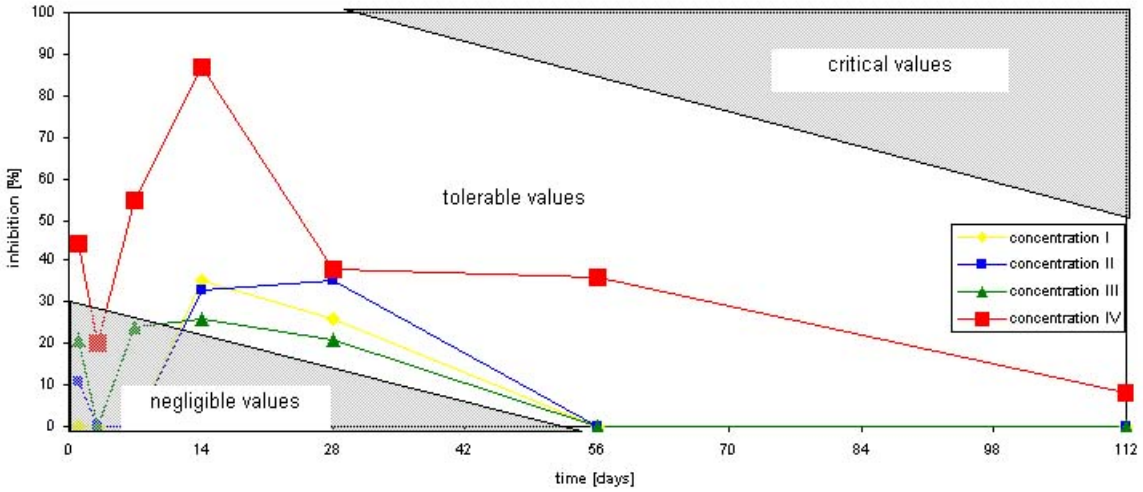
of 'tolerable values'. Low values, up to the 10% of the inhibition were registered. Though a similar trend was observed for concentration II [0.5 mM·kg^{-1}], the obtained values were higher than for concentration I. For dose III [5 mM·kg^{-1}] no significant inhibition was noted throughout the first month, however later the obtained data reached higher values from the 'tolerable' range that could significantly reduce biological transformations taking place in the soil. For concentration IV [50 mM·kg^{-1}] the highest degree of inhibition was registered. After the first month of the experiment a rapid increase in the inhibition was observed that led to a drastic disturbance of the metabolism of the soil.

Fig 2. The degree of dehydrogenase inhibition caused by various concentrations of copper (II) salt related to the ecological significance of soil environment pollution



The inhibition resulting from copper (II) salt treatment upon the soil pollution is illustrated in Fig.2. For all the concentrations inhibition stayed within the limits of 'negligible values' for the first 2 weeks of the experiment, apart from concentration III [5 mM·kg^{-1}] for which low values from the 'tolerable' range were registered. In the following period the inhibition kept growing regardless of the applied concentration. For copper (II) salt a drop in the toxic influence on the soil dehydrogenase was registered until the experiment was half way through. At the later stage, the increase of inhibition for all concentrations was registered again, with magnitudes reaching at least the limits of the 'critical values' area and most often falling within it. Hence, the conclusion can be drawn that the prolonged influence of copper (II) salt on the studied enzyme significantly contributed to slowing down the metabolic processes taking part in the soil.

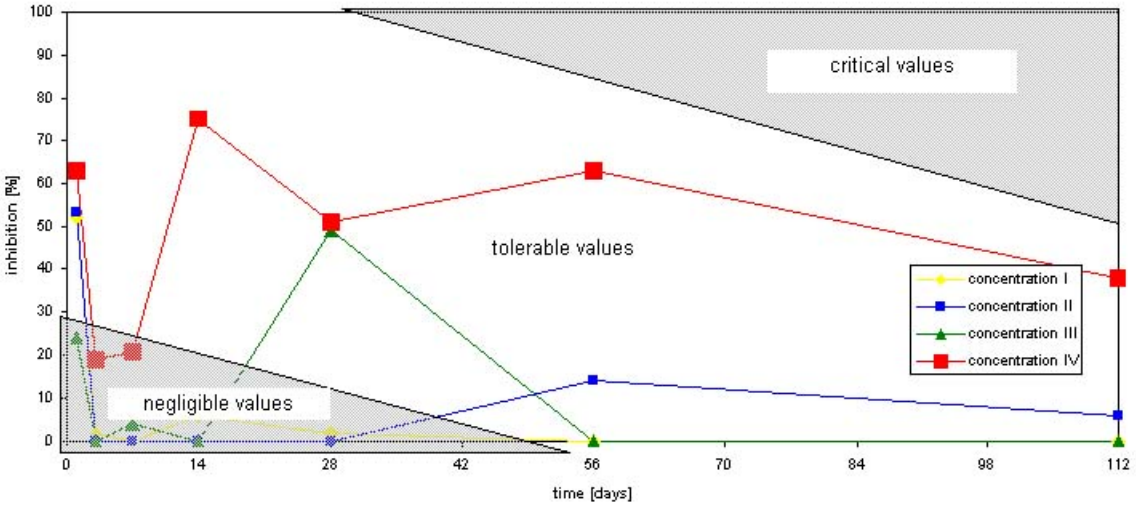
Fig 3. The degree of dehydrogenase inhibition caused by various concentrations of manganese (II) salt related to the ecological significance of soil environment pollution



The inhibition resulting from manganese (II) salt treatment upon the pollution of the soil environment is presented in Fig.3. The obtained data for concentration I [0.05 mM·kg^{-1}] indicate that despite a minor inhibition caused within the first 2 weeks of the experiment, biological life in the soil was by no means threatened. Similar trends were noted for doses II [0.5 mM·kg^{-1}] and III [5 mM·kg^{-1}] of manganese (II) salt. The influence of

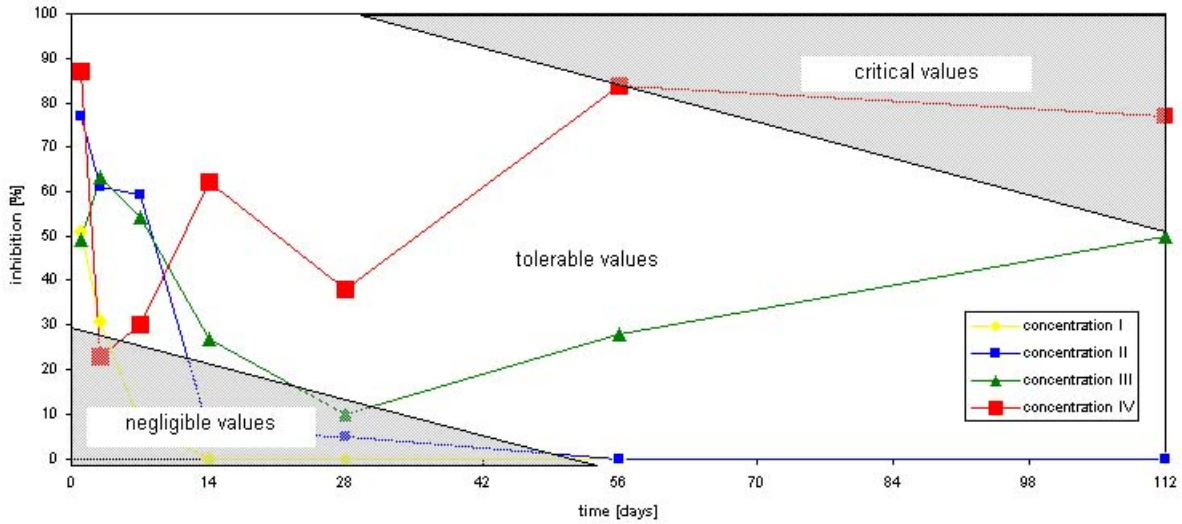
manganese salt concentration upon inhibition was found the smallest for concentration III, whereas for concentration IV [50 mM·kg^{-1}] the influence was the heaviest one. Apart from day 3 of the experiment, all the values fell within the 'tolerable' range.

Fig 4. The degree of dehydrogenase inhibition caused by various concentrations of iron (III) salt related to the ecological significance of soil environment pollution



The degree of dehydrogenase inhibition caused by various concentrations of iron (II) salts applied to the studied soil samples is presented in Fig.4. Concentrations I [0.05 mM·kg^{-1}] and III [5 mM·kg^{-1}] throughout the entire experiment did not cause the inhibition that would cause a real threat to biological life in the studied soil specimens. All the obtained data fell within the range of 'negligible values'. For concentration II [0.5 mM·kg^{-1}] a 52% inhibition was registered in the first day of the experiment, but until 56th day all the measured values were found to vary within the 'negligible values' range. Since then till the end of the experiment the enzyme inhibition caused by the influence of dose II did not endanger the microorganisms living in the soil. For the dose IV [50 mM·kg^{-1}] the highest inhibition percentage among all the applied iron (III) salt concentrations was found. Only in 3rd and 7th days of the experiment the values lay within the range of the 'negligible values', whereas for all the other days it was found to fall within the 'tolerable range' limits. The magnitude, however, has varied between 60% and 80%, hence was close to the 'critical values'. It has proved that the highest of all concentrations caused severe disturbance to the correct metabolism of microbes living in the soil.

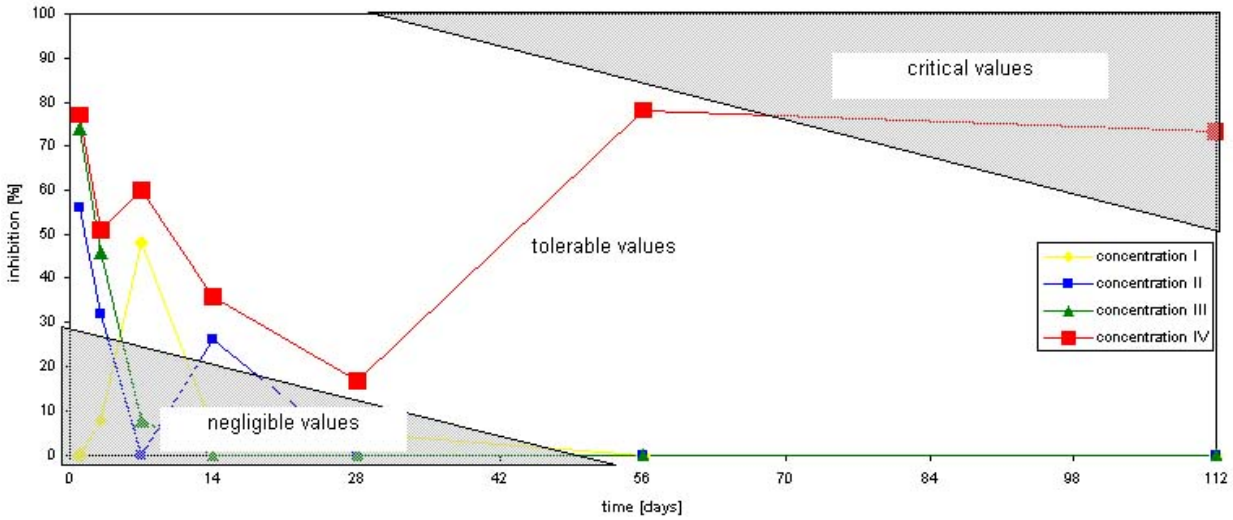
Fig 5. The degree of dehydrogenase inhibition caused by various concentrations of cobalt (II) salt related to the ecological significance of soil environment pollution



The degree of dehydrogenase inhibition caused by various concentrations of cobalt (II) salts and its influence upon the pollution of the studied soil samples is presented in Fig.5. Concentration I [0.05 mM·kg^{-1}] in the 1st and 3rd day of the experiment caused 51% and 32% inhibition, respectively, which values fell close to the lower limit

of the 'tolerable' values. After the first week of the experiment the dehydrogenase inhibition for concentration I did not affect the living organism in the soil negatively. For concentration II [0.5 mM·kg^{-1}] a similar trend was observed. Inhibition caused by concentration III [5 mM·kg^{-1}] varied within the 'tolerable values' range for almost the entire duration of the experiment. Only at the end of the first month of the research, a 10% inhibition, i.e. from the 'negligible range' was recorded. Towards the end of the experiment the values reached the 'critical' level. Hence, it can be concluded that with time progressing the increase in dehydrogenase inhibition originating from the influence of cobalt (II) salt of concentration III [5 mM·kg^{-1}], was found to be significantly disturbing to the correct transformations of the soil. For concentration IV [50 mM·kg^{-1}] a 23% inhibition of the magnitude from the 'negligible values' range was recorded only in the 3rd day of the experiment. For the rest of the days the values covered the 'tolerable values' range, and from 56th day on fell into the range of 'critical values'. Alike the other cases, the highest concentration contributed significantly to the inhibition of biological life in the soil.

Fig 6. The degree of dehydrogenase inhibition caused by various concentrations of molybdenum (II) salt related to the ecological significance of soil environment pollution



The inhibition caused by molybdenum (IV) salt treatment applied to the soil specimens and resulting soil environment pollution is depicted in Fig. 6. In general, throughout the duration of the experiment, none of the concentrations caused the inhibition that would fall into the 'negligible range'. Each of the applied doses of molybdenum (IV) salt threatened soil microorganisms, though to a different degree. Dose IV [50 mM·kg^{-1}] caused the gravest inhibition, when towards the end of the experiment the obtained data demonstrated high degree of pollution in the studied soil samples.

RESULTS AND DISCUSSION

The metals were grouped according to their significance for an undisturbed metabolism of microbes. The following groups have been defined: essential, toxic, and dispensable, e.i. Pb, as well as an additional group of 'border metals' such as Cu, Fe, Mn. Such a division can be substantiated by comparing the influence of copper (II) and lead (II) salts upon the soil dehydrogenase. While comparing the toxicity of lead (II) salt and copper (II) salts according to the suggested classification, it was proved that in the beginning stage, inhibition of the concerned enzyme was affected by Pb^{2+} ions more severely than by Cu^{2+} ions. However, after 3 month duration of the experiment, Cu^{2+} ions caused the inhibition to increase for all the concentrations applied. Cu^{2+} ions can then be used to demonstrate the aptness of classifying copper as a metal belonging to the 'border' group [7]. The enzymatic activity of soil is tightly related to the soil biological life and a number of biochemical transformations it undergoes. A heavy metal salt introduced into the soil solutions affects its microorganisms in a variety of ways. The observed changes in the activity of soil dehydrogenase originating from the heavy metal salt influence, depend on the ion concentration in the researched material, the exposition time and the type of the introduced metal. Nowak et al. confirmed such a supposition in their studies [16, 18]. From the ecological point of view, concentrations of heavy metal ions higher then the top limits specified in Polish Standards, caused significant inhibition in soil dehydrogenase activity. According to Kobus et al. [12] while determining soil dehydrogenase activity changes in the amount of microbes present in the soil are observed. Therefore taking both conclusions into consideration, it seems reasonable to suggest that the increased concentration of heavy metal ions in a soil solution results in significant inhibition of biological life.

In the field of inhibition processes and metabolic dynamics a lot of issues still remain to be explained and require further empirical research.

CONCLUSIONS

In the concluding remarks we would like to emphasize that

- The concentrations of heavy metal salts that varied within the limits of Polish Standards, namely from 0.05 $mM \cdot kg^{-1}$ to 0.5 $mM \cdot kg^{-1}$, in the beginning stage of the experiment duration caused inhibition which did not significantly threaten the micro-biological life in the studied soil.
- The top concentrations of the applied heavy metal salts of 50 $mM \cdot kg^{-1}$, caused the inhibition of the level assumed in the experiment as 'critical'. Such a high dose of heavy metal salts gravely disturbed the metabolic processes in the studied soil samples.

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Bożena Śnieg
Department of Biochemistry
Agricultural University of Szczecin
Słowackiego 17, 71-434 Szczecin, Poland
e-mail: msnieg@agro.ar.szczecin.pl

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