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Microbial Transformation of Cadmium in Two Soils Differing in Organic Matter Content and Texture

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Abstract

Results of laboratory soil suspension experiments with two soils differing in organic C content (1.1 and 4.2%) and texture (14.6 – 61.0 – 24.4% and 67.5 – 20.0 -12.5% for sand – silt – clay, respectively) showed that intensive microbial growth after the addition of a sucrose nutrient medium *(SNMed)* caused partial transformation of the solid phase Cd to forms soluble in 0.1 M NaOH. The transformation of Cd was accompanied by a simultaneous decrease of the solid phase Cd extractability by a solvent consisting of 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M triethanolamine (*DTPA*). These changes of the solid phase Cd extractability were probably caused by Cd binding to microbial biomass and microbial metabolism products. After the addition of *SNMed*, a concomitant phenomenon of an increase of Cd *DTPA*-extractability was observed, especially in the case of the course-textured soil with the higher organic C content. In a soil (not soil suspension) experiment, stimulation of microbial growth by *SNMed* addition caused different changes of Cd *DTPA*-extractability in the examined soils. In the fine-textured soil with the lower organic C content the process decreasing the solid phase Cd solubility in *DTPA* prevailed, but in the other soil, the opposite phenomenon predominated. The occurrence in nature of the phenomena observed under laboratory conditions is discussed.

Keywords: soil microorganisms, Cd extractable by NaOH, Cd extractable by DTPA, soil organic matter, soil texture.

Introduction

Pollution of agricultural land with Cd is hazardous to human health [1, 2]. The fate of the heavy metal in soil can be influenced to a great extent by microbial activity [3-8]. An understanding of these microbial factors, which both allow and prevent Cd entrance into and accumulation in the food chain of human beings, is essential.

Bollag and Czaban [9] have developed a soil suspension method that is very useful for studying the effects of microbial growth on soil Cd as a result of the obtained increased ratios of: (1) microbial biomass weight to soil weight and (2) liquid phase volume to solid phase volume. The main extractants of Cd used were: (1) a solution consisting of 0.005M DTPA + 0.01 M $CaCl_2$ + 0.1 M triethanolamine (*DTPA*) as an extractant of Cd available to plants and (2) 0.1 M NaOH (*NaOH*), as an extractant of organic Cd "strongly" bound to microbial biomass. Czaban [10] used the soil suspension method to study the influence of the intensive microbial growth (that exists in nature in the rhizosphere region or in soils enriched by organic fertilizers) on the fate of Cd in soil. The intensive microbial growth in soil suspensions enriched by various nutrient media (both acidifying and alkalizing the medium) caused considerable desorption of Cd bound by soil, and transformation of the solid phase Cd to forms soluble in *NaOH* with a simultaneous decrease of the heavy metal extractability by *DTPA* (1 *Cd-NaOH* ≈ -1 *Cd-DTPA*) [10].

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Different effects of microbial action in soils poor and rich in the organic C content on *NaOH/DTPA* solubility of the solid-phase Cd may be expected. This is because *NaOH* used by Czaban [10] to extract Cd "strongly" bound to microbial biomass has been also found to be a very good solvent of the soil humic substances [11, 12] and Cd bound to soil organic matter (*SOM*) [13, 14, 15]. In addition, Kabata-Pendias and Sadurski [16] reported that *DTPA* could extract Cd bound to *SOM*. Also, Krishnamurti *et al.* [17] found that Cd extractable with AB-DTPA was strongly correlated with the metal-organic fraction extracted by sodium pyrophosphate. Moreover, Korcak and Fanning [18] found a positive relationship between *DTPA*-extractable Cd and the amount of organic matter in soil. Similarly, a strong significant positive correlation between *DTPA*-extractable Cd and the amount of soil organic C can be calculated from the results of some other authors [19, 20, 21].

The soil used in the studies of Czaban [10] had a low organic C content, so changes of *NaOH/DTPA* solubility of the solid-phase Cd caused by copious microbial growth after the addition of the nutrient media were probably almost not disturbed by *SOM* and its possible microbial transformation, *e. g.* an increased decomposition rate – the "priming effect" [22].

It is worth mentioning that the other major soil component – the fine mineral fraction, especially after interaction with organic substances, making organic-mineral complexes, can (similarly to *SOM*) strongly influence the heavy metal distribution, mobility and bioavailability in soils [23, 24, 25, 26]. The dictum of Tan (1998), cited by Kabata-Pendias and Sadurski [16], that "various bindings between microorganisms and soil clay and humic matter seem to be of a great environmental importance," ought to be added. Therefore, the purpose of this work was to study (both in soil suspension and soil experiments) the effect of intensive microbial growth (after enrichment with a nutrient medium) on Cd *NaOH/DTPA* extractability in soils distinctly differing in organic carbon content and texture.

Materials and Methods

Characteristics of Soils

The same two soils were used as in the experiments of Bollag and Czaban [9]. They were: (1) a brown silt loam soil with low organic C content and fine texture (*LCFTsoil – Low Carbon Fine Texture soil*) (sand – 14.6%, silt – 61.0%, clay – 24.4%, org. C – 1.12%, pH in H_2O – 6.6, pH in KCl – 5.9, pH in $CaCl_2$ – 6.4, CEC <sum of Ca – 10.7, Mg – 1.1, Na – 0.08, K – 0.39 and H – 5.0> – 17.2 meq (kg) and (2) a black sandy loam soil with higher organic C content and course texture (*HCCTsoil – Higher Carbon Course Texture soil*) (sand – 67.5%, silt – 20.0%, clay – 12.5%, org. C – 4.22%, pH in H_2O – 6.6, pH in KCl – 6.1, pH in $CaCl_2$ – 6.4, CEC <sum of Ca – 13.0, Mg – 3.9, Na – 0.06, K – 0.29 and H – 6.5> – 23.7 meq (kg) [9].

Influence of Microbial Biomass Content and Microbial Activity on the Fate of Cd in Soil Suspensions

To prepare the 5% soil suspension *(5% SSusp)*, 2.5 g of the soils (passed through a 2 mm sieve) were put into 125 cm^3 Erlenmeyer flasks with 50 cm^3 of phosphate buffer *(PhBuf)*, containing 4.8 g K_2HPO_4 and 1.2 g KH_2PO_4 in 1 dm^3 (pH 7.3). This amount of the soils was the smallest, which almost totally sorbed Cd from the solution. The suspensions were preincubated with Cd (10 (μg/cm^3) as $CdCl_2$ on a rotary shaker at 27°C for 24 hours. After this, 2 cm^3 of dist. H_2O (control) or a concentrated sucrose nutrient medium *(SNMed)* was added, to reach a final concentration of the following nutrients: sucrose – 1%, NH_4NO_3 – 0.05% and $MgSO_4$ x $7H_2O$ – 0.02% (w/v).

The samples were taken from separate flasks after 1 hour and 1, 2, 3, 4 and 6 days of the incubation at 27°C on a rotary shaker for the determination of pH and dehydrogenase activity, and after centrifugation at 1000 x g for 10 min. for the determination of Cd content in the supernatants and the pellets as well as in *NaOH* and *DTPA* extracts of the pellets.

Influence of Microbial Biomass Content and Microbial Activity on the Fate of Cd in Soils

In this experiment, 50 g subsamples of the soils (sieved through a 2 mm screen) were placed in 125 cm^3 jars (12 jars for each kind of soil). Cd was added into each jar to make the final concentration of the heavy metal of 10 μg per 1g of soil. The jars were divided into two series. In one series, a nutrient solution was added to the soil to reach a final concentration of the following nutrients: sucrose – 2%, NH_4NO_3 – 0.1%, K_2HPO_4 – 0.1% and $MgSO_4$ – 0.05%. Into the other, control series distilled water was added. The water content of the soils was maintained at 50% of their water holding capacity. Soil samples of approximately 3 g were taken from each jar after 30 min, 1, 2, 3, 4, 6, 9, and 15 days of incubation at 27°C. The samples taken from the jars of each individual series were mixed together. The four 1 g subsamples were transferred into centrifuge tubes for extraction of Cd. The other 2 g subsamples of soil were taken for determination of pH (soil: H_2O dist. = 1: 2 <w/v>) and dehydrogenase activity.

Extraction of Cd and Determination of Its Concentration

Cd from 1 g samples of the soils or from the remaining pellets obtained after centrifugation of 10 cm^3 sam-

ples of the soil suspensions was extracted (after triple rinsing with 5 cm^3 of dist. H_2O) in two different ways: (1) three times with 5 cm^3 of 0.1 M NaOH (*NaOH*) for 15 min and then three times with 5 cm^3 of 1 M HNO_3; and (2) three times with 5 cm^3 of a solution (pH 7.3) containing: 0.005 M diethylenetriaminepentaacetic acid, 0.01 M $CaCl_2$, and 0.1 M triethanolamine (*DTPA*) for 15 min and then three times with 5 cm^3 of 1 M HNO_3. In the soil suspension experiment Cd was additionally extracted with *NaOH* after previous extraction with *DTPA*. Preparation of the supernatants and the extracts for the determination of Cd concentration was done according to Bollag and Czaban [9]. Cadmium concentrations were determined by atomic absorption spectrophotometry.

Dehydrogenase activity was measured according to Bollag and Czaban [9].

Statistical Evaluations

For estimation of all examined relationships a simple correlation analysis was used. Correlation coefficients (r), probability (p) and the number of values (n) are presented. For evaluation of the relationship between the changes of Cd extractability with *NaOH* and the changes of Cd extractability with *DTPA*, regression analysis was applied. This relationship was determined using results of the series enriched with *SNMed* from which the control values of the soil suspensions not enriched by *SNMed* were subtracted. Together with the linear equations, regression coefficients (R^2) are presented.

Results and Discussion

Influence of the Intensive Growth of Microorganisms on the Fate of Cd in the Soil Suspensions

In the series not enriched by *SNMed*, 82-87% of Cd was sorbed by the soils from the liquid medium after 30 min, and more than 98% during further incubation (Fig. 1C). After 30 min, more than 90% of the solid phase Cd were *DTPA*-extractable, but after 6 days the extractability dropped to about 70 and 80% in the case of *LCFTsoil* and *HCCTsoil*, respectively (Fig. 1D). A similar decrease of the solid phase Cd extractability by *DTPA* in *5% SSusp* was observed by Bollag and Czaban [9] and Czaban [10]. *NaOH* could remove more Cd sorbed by *HCCTsoil* than from *LCFTsoil*. It suggests that this part of Cd was bound to *SOM* (Fig. 1E and 1F).

The addition of *SNMed* caused the appearance of the intensive dehydrogenase activity (Fig. 1B), and acidification of the medium (Fig. 1A), indicating copious growth of microorganisms and the transformation of sucrose to organic acids. The decrease of pH was accompanied by a 6% desorption of Cd bound to the soils (Fig. 1C). The desorption of Cd was positively correlated with the acidification of the medium ($r = 0.840$, $p < 0.05$, $n = 6$ for *LCFTsoil*, and $r = 0.969$, $p < 0.01$, $n = 6$ for *HCCTsoil*).

SNMed addition caused a significant increase in the proportion of solid phase Cd extractable by *NaOH* (Fig. 1E and 1F). This higher *NaOH*-extractability of Cd was accompanied by a decrease of the solid phase Cd extractability by *DTPA* (Fig. 1D). The changes of Cd *NaOH*-extractability were significantly negatively correlated with the changes of Cd extractability by *DTPA* ($r = -0.962$ at $p < 0.01$, $n = 6$ for *LCFTsoil*, and $r = -0.943$ at $p < 0.01$; $n = 6$ for *HCCTsoil*).

Solid phase Cd was extracted with *NaOH* and *DTPA* from separate samples to prevent interference between the solvents. In order to show that *DTPA* can not extract all of the Cd that is soluble in *NaOH*, an additional extraction of Cd with *NaOH*, after previous Cd extraction with *DTPA*, was performed. Similarly to the direct Cd extraction by *NaOH*, amounts of Cd *NaOH*-extractable after the previous Cd extraction by *DTPA* from the experimental series enriched by *SNMed* were also distinctly higher than the corresponding control values (Fig. 1E and 1F). After subtracting the corresponding control values, the concentrations of *Cd-NaOH* after previous extraction by *DTPA* ranged between 40-70% of those amounts of *Cd-NaOH* directly extracted by *NaOH*.

In the case of the experimental series not enriched by *SNMed*, *NaOH* could remove more Cd (especially in the case of the *LCFTsoil*) after the previous extraction of the element by *DTPA* than from the soil suspension not treated with *DTPA* (Fig. 1E and 1F). This progressive increase of Cd extractability by *NaOH* was related to the progressive decrease of Cd extractability by *DTPA* (Fig. 1D). A significant negative correlation ($r = -0.974$ at $p < 0.01$; $n = 5$ – for both kinds of soil) between the changes of Cd *DTPA*-extractability and the changes of Cd *NaOH*-extractability was found. This suggests that during the extraction of Cd by *DTPA* extractant, DTPA was fixed to Cd previously bound to the soil or the soil (mainly clay fraction) fixed DTPA-Cd complex. Later, *NaOH* could remove from the soil this part of Cd attached to *DTPA*. Norvell and Lindsay [27] reported rapid losses of DTPA chelates of various metals due to adsorption on particles of silt loam and loam soils. The progressively increased ability of the soils to bind *DTPA* (or/and *DTPA-Cd*) was probably connected with a gradual increase in the formation of Cd-phosphate-soil surface complexes. The possibility of the formation of such ternary complexes, where both metal and phosphate could be bonded to the surface, was reported by McBride [28]. The study of Panvar and Morvai [29] confirms this assumption. They found that Cd and phosphates induce the fixation of each other in a loamy sandy soil, and that the Cd extractability by *DTPA* and phosphate extractability by Olsen's solvent decreased with increasing incubation period. Also, Bolan *et al.*[30], Naidu *et al.*[31] and Xiong [32] reported that phosphate treatment increased the soil adsorptive strength for Cd through the increased negative surface

charge, and Gray *et al.* [33] found that the total soil Cd concentrations were highly correlated with total soil phosphorus.

The phenomenon of changes of the solid phase Cd solubility in *NaOH* and *DTPA* during the copious growth of microorganisms was most likely connected with Cd binding to the microbial biomass. Bollag and Czaban [9] found in the 0.05% soil suspension *(0.05% SSusp)* experiment that the copious microbial growth was accompanied by the disappearance of Cd from solution, a considerable increase of the solid phase Cd extractability by *NaOH* and a strong decrease of its *DTPA*-extractability. In this experiment, the small amount of soil was used mainly as the source of microorganisms, so it could not disturb the determination of the microbial biomass, that was proliferating in the soil suspension after addition of *SNMed*. Some

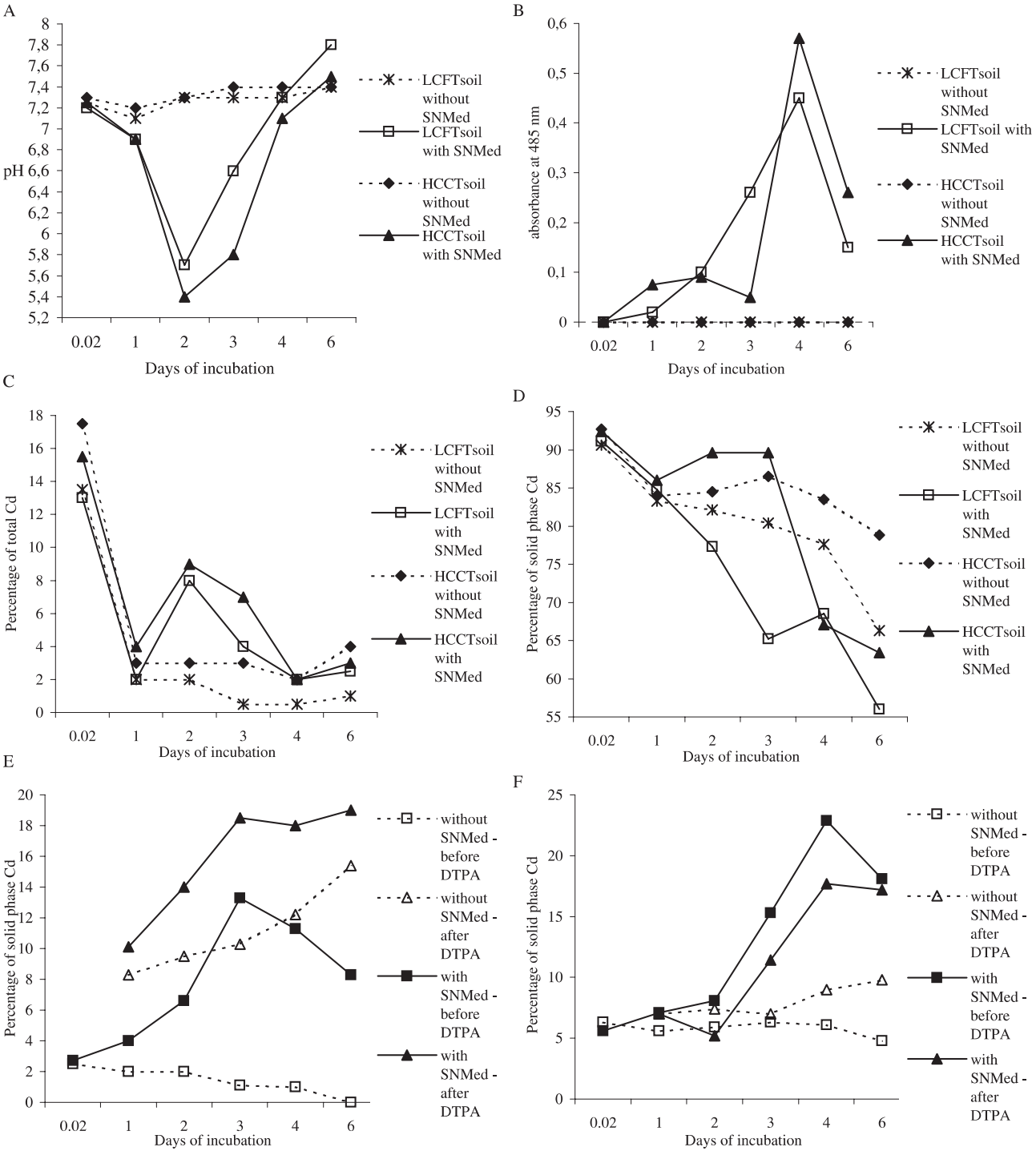

Fig. 1. Effect of intensive microbial growth in 5% soil suspension experiment on: (A) pH; (B) Dehydrogenese activity; (C) Liberation of solid phase Cd into liquid phase; (D) Changes of solid phase Cd extractability by *DTPA*; (E) Changes of solid phase Cd extractability by *NaOH* in *LCFTsoil* suspension; (F) Changes of solid phase Cd extractability by *NaOH* in *HCCTsoil* suspension.

results obtained from the *0.05% SSusp* experiment of Bollag and Czaban [9] (a part of them – *e. g.* the results concerning the solid phase Cd *NaOH/DTPA*-extractability – was not presented previously) are shown in Fig. 2. The present calculations, made on the basis of results of Bollag and Czaban [9], show that the microbial biomass was significantly (at $p < 0.05$; $n = 6$) positively correlated with: the total sorbed Cd ($r = 0.958$ and 0.911) and the changes of Cd *NaOH*-extractability ($r = 0.971$ and 0.988), and negatively correlated with the changes of Cd *DTPA*-extractability ($r = -0.874$ and -0.870) in the case of *LCFTsoil* and *HCCTsoil*, respectively. The changes of sorbed Cd extractability by *NaOH* was negatively correlated with the changes of its *DTPA*-extractability ($r = -0.894$ and -0.945).

Besides the fixation of Cd to microbial biomass, the binding of Cd to various microbial metabolic products is also possible. Kurek *et al.* [34] found that an extracellular protein from *Arthrobacter* sp. spent medium formed a water-insoluble precipitate with Cd, and Fischer and Bipp [11] observed an increase of heavy metal (including Cd) extraction from soil under strongly alkaline conditions after the addition of natural chelating agents – D-gluconic or D-glucaric acids – the products of microbial sugar transformation.

During the first three days of the incubation of *5% SSusp* enriched by *SNMed*, a distinct difference between the soils was observed, especially after subtracting the values of the control series (Fig. 3A and 3B). In the case of *HCCTsoil*, extractability of the sorbed Cd by *DTPA* increased significantly (Fig. 3B). At that time a similar, but much smaller increase of Cd solubility in *DTPA* was detectable in the case of *LCFTsoil* (Fig. 3A). A yet more distinct difference in the Cd *DTPA*-extractability between the two examined soils was evident in the similar *5% SSusp* experiment of Bollag and Czaban [9], which was performed with the same soils and with the same microbial nutrient medium. However, the authors have not mentioned differences in Cd extraction by *DTPA* between these two soils. The transformed (after subtracting the control values) data of their *5% SSusp* experiment are presented in Figs. 3C and 3D. The increase of *DTPA*-extractability of the sorbed Cd in the case of *HCCTsoil*, occurring at the beginning of the incubation period, was much more significant than that of *LCFTsoil* and it lasted longer. The changes of Cd extractability by *NaOH* were significantly negatively correlated with the changes of Cd *DTPA*-extractability ($r = -0.941$ at $p < 0.01$; $n = 6$)

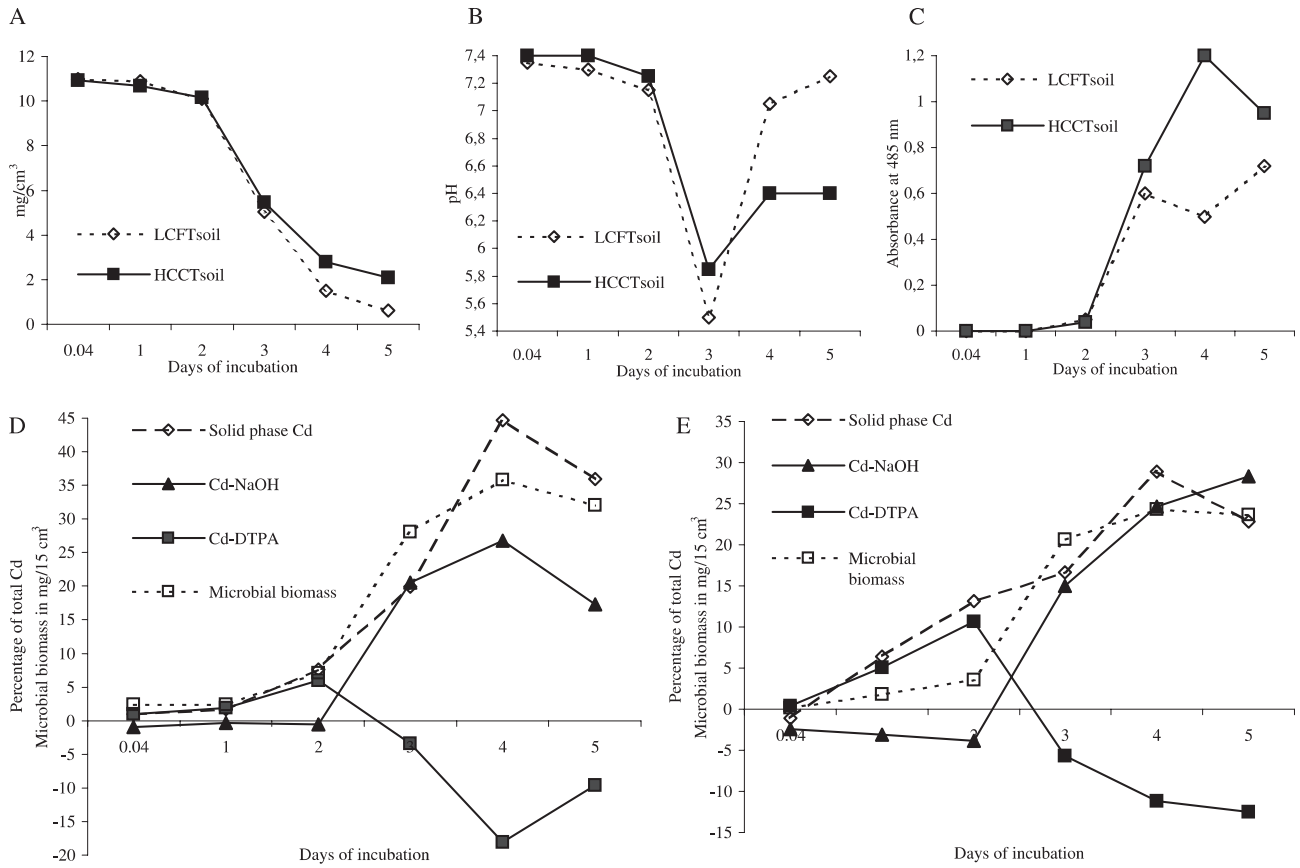

Fig. 2. Disappearance of nutrients (A), pH changes (B), dehydrogenase activity (C), as well as weight of microbial biomass and transformation of liquid phase Cd to solid phase Cd and its forms soluble in *NaOH* and *DTPA* in 0.05*% Ssusp* of *LCFTsoil* (D) and *HCCTsoil* (E) enriched with *SNMed*. Figures D and E present transformed (after subtracting the control values of the series without nutrients added) results of experiment performed by Bollag and Czaban [9]. Results concerning the disappearance of nutrients, pH changes and extractability of Cd with *NaOH* and *DTPA* were not previously presented in any figures or tables.

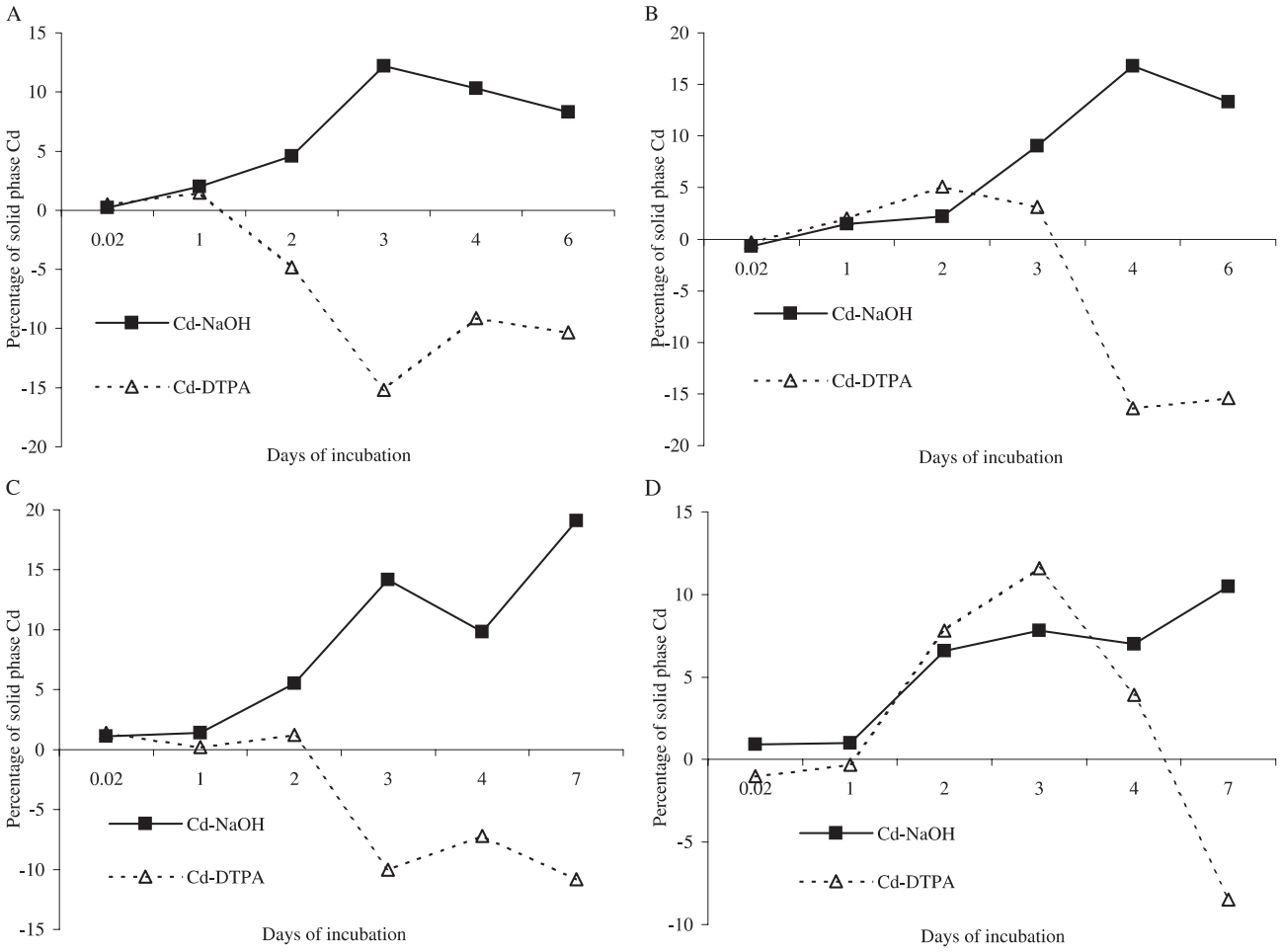

Fig. 3. Effect of intensive microbial growth on solid phase Cd extractability in 5% soil suspension experiments of the present study: (A) – *LCFTsoil* suspension and (B) – *HCCTsoil* suspension, and of study of Bollag and Czaban [10]: (C) – *LCFTsoil* suspension and (D) – *HCCTsoil* suspension. All results presented are obtained after subtracting the control values (without nutrients added).

only in the case of *LCFTsoil*. The results of both *5% SSusp* experiments (the present one and that of Bollag and Czaban [9]) suggest that some transformations/decomposition of *SOM* could be, among other phenomena, responsible for these increases of solid phase Cd solubility in *DTPA*. Schauer *et al.* [35] reported that decomposition of sewage sludge in soil resulted in significant increases in *DTPA*-extractable metals, including Cd. The importance of microbial activity in the release of trace elements from metalliferous peat soils was emphasized also by Qureshi *et al.* [36].

It would seem that the changes of the solid phase Cd solubility in *DTPA* in *5% SSusp* could be connected with the acidification of the medium that occurred after the second and third day of the incubation (Fig. 1C). However, similar increases of the solid phase Cd extractability by *DTPA* also were observed after one or two days of incubation in *5% SSusp* in the experiments of Czaban [10] (performed with *LCFTsoil*), where besides glucose and starch (acidification of the medium occurred), nutrient broth or peptone with yeast extract (alkalization of the medium occurred) were used as the nutrient media (Fig. 4).

A similar phenomenon was observed in the *0.05% SSusp* experiment of Bollag and Czaban [9] in the suspension of both soils enriched by *SNMed*. Some data from this experiment are shown in Fig. 2. On the 2nd day of the incubation, when microbial growth was not yet substantial, 7 and 13% more of the total Cd (in compari-

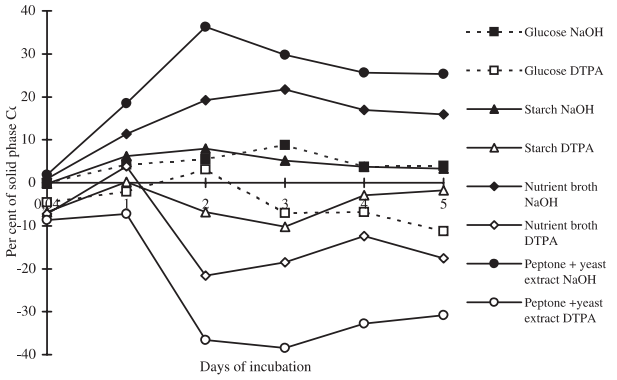

Fig. 4. Effect of intensive microbial growth on solid phase Cd extractability by *NaOH* and *DTPA* in 5% LCS suspension experiment of Czaban [11]. All results presented are obtained after subtracting the control values (without nutrients added).

son with the corresponding control series) was shifted from the solution phase to the solid phase in the suspension of *LCFTsoil* and *HCCTsoil*, respectively (Fig. 2). This solid phase Cd was almost completely extractable by *DTPA* and not by *NaOH* (Fig. 2D and 2E). It is certain that the microbial action was responsible for the observed appearance of the solid phase Cd soluble in *DTPA*. Incubation of the soil suspensions with *SNMed* in sterile conditions did not cause such changes of Cd (results are not shown). However, it is not clear whether some microbial transformations/decomposition of *SOM* caused the doubling in shift of soluble Cd to solid phase Cd in *0.05% SSusp* of *HCCTsoil* in comparison with that in *0.05% SSusp* of *LCFTsoil* (as it was supposed earlier) because the microbial activity was very low at the time (Fig. 2). It seems that the formation of some precipitates soluble in *DTPA* is also possible. Sauvé [37] mentioned after Schalscha *et al.* (1982) that DTPA and EDTA dissolved metal "precipitates", and some other scientists used EDTA for extraction of the cadmium carbonate fraction [13, 15, 19]. It should be mentioned that sorption of Cd by both soils not enriched by *SNMed* was very similar. It increased from about 30% of the total Cd after 1h to about 40% at the end of the 5 day-incubation period (results are not shown).

Influence of the Intensive Growth of Microorganisms on the Fate of Cd in the Soil Experiment

Cd was almost totally sorbed by the soil particles. Water as an extractant could remove only 0.5-2.7% and 1.1-2.2% of the total Cd content from *LCFTsoil* and *HCCTsoil*, respectively (results are not shown). At the beginning of the 15-day incubation period, the pH of the soils was close to neutral (6.8 and 7.0 of *LCFTsoil* and *HCCTsoil*, respectively). During further incubation, in the control (not enriched by the nutrients) series, the pH of soil gradually increased to 7.2 and 7.6 in the case of *LCFTsoil* and *HCCTsoil*, respectively. In comparison with the control, nutrient addition decreased the pH of the soils by 0.4-0.5 of a unit during the whole incubation period (results are not shown).

In the control series, the dehydrogenase activity was about 10 times greater in *HCCTsoil* than in *LCFTsoil*. After the addition of nutrients, the dehydrogenase activity increased 25-times in *LCFTsoil* and only 4-times in *HCCTsoil*. This suggests that the organic nutrient pool available to microorganisms was much higher in *HCCTsoil* than in *LCFTsoil* (results are not shown).

In the control series, at the beginning of the incubation period, *NaOH* removed 4% and 10% of the solid phase Cd from *LCFTsoil* and *HCCTsoil*, respectively. It suggests that this Cd fraction was connected with *SOM*. Further incubation caused a progressive decrease of *Cd-NaOH* content by about 40% in both soils (Fig. 5A). The addition of nutrients caused an increase in the *Cd-NaOH* proportion. The greatest difference (observed after 1 and 2 days of the incubation in the case of *LCFTsoil* and *HCCTsoil*, respectively) in *Cd-NaOH* content between the series enriched by the nutrients and the corresponding control series exceeded 3% of the solid phase Cd in both studied soils. This *Cd-NaOH* fraction decreased during further incubation to less than 0.5% of the solid phase Cd (Fig. 5A, 5C and 5D). This suggests that this form of Cd, which was most likely bound to microbial biomass, was readily degradable. A similar conclusion can be drawn from results of Chanmugathas and Bollag [38], who observed immobilization and subsequent mobilization of Cd in a soil suspension enriched by a microbial nutrient medium.

Bollag and Czaban [9] found that clay and a silt loam soil could interfere with *NaOH*-extraction of Cd sorbed by the bacterial biomass. These substances, mixed with bacterial biomass containing Cd just before the extraction, considerably reduced (through the adsorption on their surfaces) the *NaOH*-extractability of Cd. This suggests that in the present soil experiment the amount of microbial Cd soluble in *NaOH* might have been bigger than the determined 3% of solid phase Cd.

In the control series at the beginning of the incubation period, *DTPA* extracted more than 90% of the sorbed Cd by both kinds of soil. During further incubation the extractability decreased to about 70-75% (Fig. 5B). The addition of nutrients to *LCFTsoil* caused a further (max. by about 10% of the total solid phase Cd) decrease of the solid phase Cd extractability by *DTPA* (Fig. 5B and 5C). The opposite trend occurred with the *HCCTsoil*. In this soil, stimulation of microbial growth by the addition of nutrients increased (max. by about 5% of the total solid phase Cd) the sorbed Cd extractability by *DTPA* for the whole incubation period (Figs. 5B and 5D). The simultaneous increase of the solid phase Cd extractability by *DTPA* with the decrease of Cd extractability by *NaOH* in *HCCTsoil* suggests that some part of *Cd-DTPA* might have come from the microbial *Cd-NaOH* decomposition (Fig. 5D).

No statistically-significant correlation between the changes of Cd extractability with *NaOH* and the changes of Cd extractability with *DTPA* under the microbial influence was found with either soil. Although Fig. 5C showed that in the case of *LCFTsoil* the highest peak of *Cd-NaOH* was accompanied by the lowest point of *Cd-DTPA*.

It should be remembered that similar differences in changes of *DTPA*-extractability of Cd under microbial action between the two soils were also observed (but only in the first few days of the incubation) in the soil suspension experiments (Figs. 2 and 3), when the microbial biomass content was still small. These results from the soil suspension experiments suggest that during the intensive growth of soil microorganisms at least two different microbial processes were involved in changing the solid phase Cd. The first process decreased the solid phase Cd extractability by *DTPA* (and probably Cd availability to

plants) due to "strong" binding with the microbial biomass and with microbial metabolism products. The possibility of sorption by clay should also be considered. The other process (or rather processes) which increased the solid phase Cd extractability by *DTPA* (and maybe Cd availability to plants, because higher *DTPA*-extractability of Cd does not always mean greater availability to plants [39, 40, 41]), was possibly due to, among other things, some microbial transformation/decomposition of *SOM*. In *LCFTsoil* the former process predominated but in *HCCTsoil* the latter phenomenon prevailed. It may be expected that such phenomena exist in natural conditions, e. g. after the addition of sewage sludge or manure to soils and in the rhizosphere region, where the soil is enriched by organic root exudates that are readily available to the microorganisms. The results of Gaynor and Halstead [20] show that this is not pure supposition. They observed distinct increases (in comparison with non-incubated soils amended with the sewage sludge) of solid phase Cd extractability by *DTPA* after incubation of sewage sludge in two sandy loam soils – one rich and the other poor in organic matter (*OM*) content, and a distinct decrease of Cd extractability by *DTPA* in a clay soil, poor in *OM*. Moreover, they observed in the soils enriched by the sewage sludge after cropping to lettuce, a yet more distinct increase of Cd extractability by *DTPA* in the sandy loam soil, rich in *SOM*, and a yet more distinct decrease of Cd extractability by *DTPA* in the clay soil [20]. The plants grown on the soils enriched by both the sewage sludge and mineral fertilizers contained (in comparison with the plants grown on the soils with only mineral fertilizers) more Cd in the case of the sandy loam soil rich in *OM*, and less Cd in the case of the clay soil [20]. Perhaps the same microbial mechanisms, influencing the *DTPA*-extractability of Cd, were working both in the study of Gaynor and Halstead [20] and in the present study.

As with the *HCCTsoil* in the present study – a distinct increase of Cd extractable by *DTPA* – was also observed (in comparison with the bulk soil) by El-Motaium and Badawy [42] in the rhizosphere of cabbage and orange trees, irrigated with sewage water. In contrast, Hammer and Keller [43] found that the concentrations of Cd extractable with *DTPA* in the rhizosphere of common osier in an acid soil and in the rhizosphere of *Thlaspi caerulescens* in acid and calcareous soils were lower than

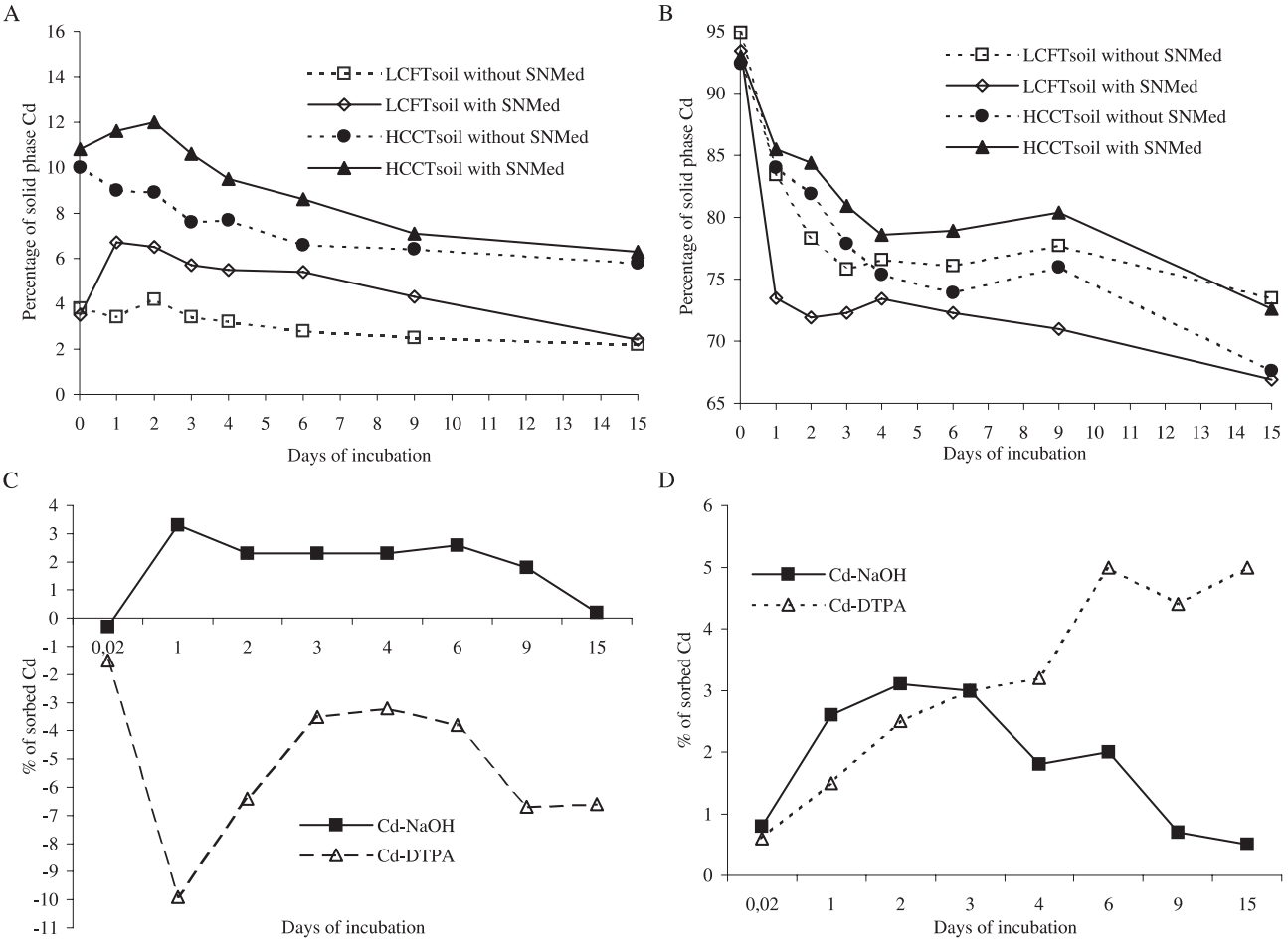

Fig. 5. Effect of intensive microbial growth in soil experiment on changes of solid phase Cd extractability by *NaOH* (A) and *DTPA* (B). The lower figures present the changes of Cd extractability by *NaOH* and *DTPA* between the series enriched with the nutrients and not enriched control (the control values are subtracting) in *LCFTsoil* (C), and *HCCTsoil* (D).

in the corresponding non-rhizosphere soils. The present calculations made on the basis of the results of Hammer and Keller [43] show that the uptake of Cd by plants may have been responsible for the depletion of *Cd-DTPA* concentration in the rhizosphere of osier by only 5%, and in the rhizosphere of *T. caerulescens* – the Cd hyperaccumulator grown on the calcareous soil by 25%. Similarly, Lorenz *et al.* [44] found that concentrations of Cd in the solutions of radish rhizosphere grown on ten soils were lower than those in the solution of the corresponding non-rhizosphere soils and contained higher proportions of complexed forms of Cd. Lorenz *et al.* [44] also found that Cd concentration in the plants was more strongly positively correlated with the concentration of Cd in the rhizosphere solutions ($R^2 = 0.98$) than with the Cd concentration in the solution of non-rhizosphere soils ($R^2 = 0.50$). Moreover, on the basis of new calculations made on the results of Lorenz *et al.* [44], the Cd concentration in the plants was not correlated with the difference in Cd concentration in the soil solution between the non-rhizosphere and rhizosphere soils ($R^2 < 0.07$). It may be concluded that the depletion of Cd concentration in the rhizosphere solution was mainly caused by other processes (e. g. microbial action) rather than by uptake of the metal by plants.

Comparison of Relationships Between the Changes of Solid Phase Cd Extractability by NaOH and DTPA in Various Experiments

Fig. 6 presents linear relationships between the changes of the solid phase Cd solubility in *NaOH* and *DTPA* in: the *0.05% SSusp* (Fig. 6A), the *5% SSusp* (Fig. 6B) and the soils (Fig. 6C). The calculations were made on the basis of results of the present study (*5% SSusp* and soil experiments) and the results obtained by Bollag and Czaban [9] (*0.05 SSusp* and *5% SSusp* experiments), because Bollag and Czaban [9] used the same soils and the same sucrose nutrient medium in their experiments.

In the case of the *0.05%SSusp* experiment the linear trends for both soils are in almost the same position with only a very small shift of the *HCCTsoil* line to higher values of *Cd-DTPA*. The regression coefficients (R^2) values are very high for both soils, which shows that in both soil suspensions the process of binding Cd to microbial biomass and microbial metabolism products mainly occurred (Fig. 6A). The trend lines of in the case of *5%SSusp* are more separated, but they are almost parallel, which means that the process of Cd binding to microbial biomass occurred in both soil suspensions at similar rates (Fig. 6B). The much lower R^2 value in the case of *HCCTsoil* shows that the concomitant microbial process/processes increasing the *DTPA*-extractability of Cd disturbed the former phenomenon to a greater extent. In the case of the soil experiment the lines are widely separated (Fig. 6C). This means that in the different soils different phenomena prevailed. These results suggest that the observed effect of the various microbial processes, increasing or decreasing Cd extractability with *NaOH* and *DTPA*, depended in the experiments on the ratio of microbial biomass to mass of soil. This ratio was much greater in the soil suspensions than in the soils, so the phenomenon of decrease of solid phase Cd solubility in *DTPA*, which was connected with microbial Cd transformation to forms extractable by *NaOH*, could be detected in the case of *HCCTsoil* only in the soil suspension experiments.

Conclusions

1. Intensive microbial growth after the addition of a sucrose nutrient medium caused a partial transformation of solid phase Cd to forms soluble in 0.1 M NaOH in both a brown silt loam soil with low organic C content and a black sandy loam soil with high organic C content. The process most likely occurred due to the binding of the heavy metal to microbial biomass and products of microbial metabolism.
2. With the brown silt loam, the transformation of Cd described above was accompanied by simultaneous decrease of the solid phase Cd extractability by a solvent consisting of 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M triethanolamine both in soil suspension and soil experiments.
3. With the black sandy soil, the phenomenon of de-

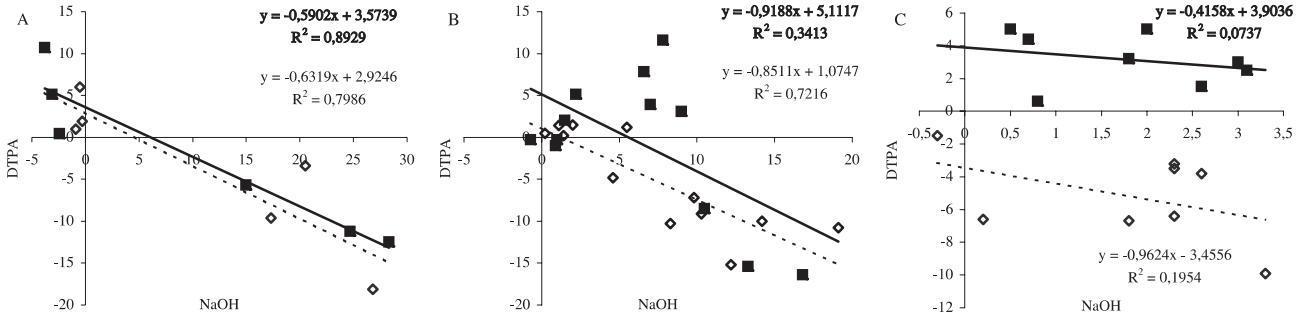

Fig. 6. Relationship between changes (under the influence of the microorganisms) of Cd extractability by *NaOH* and *DTPA* in: (A) the 0.05% soil suspensions, (B) the 5% soil suspensions and (C) in the soils. The empty squares, thin line and the equation in normal font concern *LCFTsoil*. The filled squares, thick line and the equation in bold font concern *HCCTsoil*.

crease of Cd extractability by the solvent containing DTPA could be observed only in soil suspensions because of the high ratio of microbial biomass to mass of soil. In the soil experiment the opposite phenomenon prevailed probably because of microbial transformation of soil organic matter bound to Cd.

4. On the basis of literature cited, the phenomena of increase or decrease of Cd extractability by DTPA due to microbial action, observed under laboratory conditions, can be expected to occur also in nature, e. g. after the addition of organic fertilizers to soil and in the rhizosphere region of plants.

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