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Original Research

Microbial Transformation of Cadmium Sorbed by Soil

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Abstract

Intensive microbial growth on 4 nutrient media (containing glucose, starch, nutrient broth or peptone + yeast extract) in soil suspension experiments, changing pH (acidification or alkalization) caused partial liberation of Cd sorbed by soil particles to liquid phase. At the same time a considerable transformation of the solid phase Cd to soluble in 0.1 N NaOH form was observed. Increased Cd concentration in the alkaline extract was accompanied by decrease of the heavy metal extractability with DTPA. The mechanisms which might be responsible for the observed phenomena are discussed. The obtained results suggest that microbial action can simultaneously increase and decrease availability of Cd to plants.

Keywords: soil-sorbed Cd, soil microorganisms, NaOH-extractable Cd, DTPA-extractable Cd, Cd desorption

Introduction

Cadmium, a heavy metal with an unknown essential biological function, is one of the most toxic pollutants of the environment [13, 27, 35]. The accumulation of Cd in soils has become a major concern for food production. Cd concentration in plants is related to the concentration of its forms available to plants [13, 27]. The bioavailability of cadmium in soils is dependent on the partition of the metal between the solid and liquid phases. The sorption of the metal by surfaces of organic and inorganic soil constituents is largely responsible for the partition [19, 24, 26, 30]. Microorganisms, with their large surface area relative to their volume, and with relatively high cation exchange capacities, are potentially an important organic soil constituent, with very high capacity for sorbing metals from solution [14, 16, 17, 24, 26, 28]. The partition of Cd in soil between the two phases is also strongly influenced by various other microbial activities e.g. changing of soil pH, production of soluble organic substances which can chelate Cd, or the formation of various precipitates [4, 11, 18, 30].

The most important factor influencing the solubility and mobility of Cd, as well as its availability to plants appears to be pH [8, 12, 13, 23, 27, 30, 33, 34]. Microbial transformation of organic compounds existing in soil, especially in the rhizosphere region, may acidify (through production of organic acids) or alkalize (through ammonification) surrounding space [31, 32]. This means that different changes of pH are possible, depending on the source of organic C.

Bollag and Czaban [6] have developed a soil suspension method which enables us to obtain exaggerated ratios of weight of microbial biomass to soil weight and of liquid phase volume to solid phase volume. Such a method is very useful for studying the effect of microbial growth on soil Cd.

The purpose of this paper is to use this method to study the outcome of Cd sorbed by soil particles in soil suspensions, which were enriched with various nutrient media, where intensive microbial growth causes acidification or alkalization of the medium.

Materials and Methods

Soil

A silt loam soil (pH_{water} 6.6; pH_{KCl} 5.9; cation exchange capacity 17.2 meq kg^{-1}; Ca 10.7 meq kg^{-1}; org-C 1.1%; clay 24.4%; silt 61.0%; sand 14.6%) was used in the experiments.

The three following experiments were performed: I, II and III.

(I) Studies of Fate of Cd Sorbed by the Soil Particles in Non-Sterile Conditions (Experiment I)

The soil (2.5 g) was suspended in 50 cm^3 of 0.036 M phosphate buffer (K_2HPO_4 - 4.8 g, KH_2PO_4 - 1.2 g, dist. H_2O - 1000 cm^3, pH - 7.3), with 10 μg cm^{-3} Cd as $CdCl_2$ added. The soil suspension was preincubated on a rotary shaker with Cd for 24 hours at 30˚C to bind Cd to the soil particles. Then, to control series (1), 2 cm^3 of dist. H_2O, and to other series (2, 3, 4, 5) 2 cm^3 of concentrated media were added. Afterwards, five experimental series were established:

(1) control - the soil suspended only in the buffer (Bu),
(2) as 1 supplemented with 1% (w/v) of glucose (Gl),
(3) as 1 supplemented with 1% (w/v) of soluble starch (St),
(4) as 1 supplemented with 1% *(w/v)* of Nutrient broth - Difco (Nb),
(5) as 1 supplemented with 1% (w/v) of a mixture (l:lw/w) of Difco peptone + yeast extract (PY).

Both "carbohydrate nutrient media" (Gl and St) contained 0.05% of NH_4NO_3 and 0.02% of $MgSO_4$ x $7H_2O$ (w/v).

After 1 hour, and 1, 2, 3, 4 and 5 days of incubation at 30°C on the rotary shaker, samples of the soil suspensions of all 5 series were taken for determination of pH and dehydrogenase activity (a microbial growth and activity indicator) as well as for determination of Cd concentration in various fractions. Separate flasks were taken for each time of sampling. All procedures were performed according to Bollag and Czaban [6]. The estimation of dehydrogenase activity was based on the use of 2,3,5-triphenyltetrazolium chloride as an artificial electron acceptor. After centrifugation of the soil suspension, "desorbed Cd", liberated into liquid phase, was determined in supernatant. After washing the precipitate (obtained by centrifuging) with distilled water, "sorbed Cd" present in solid phase, was extracted with two separate systems [6]:

(1) with 0.1 M NaOH followed by 0.1 M HNO_3,
(2) with 0.005 M. DTPA supplemented with 0.01 M CaCli and 0.1 M triethanolamine followed by 0.1 M HNO_3.

The NaOH solution was chosen as an extractant of some part of Cd sorbed by microbial cells, and the DTPA solution as an extractant of "available to plants" form of Cd [6]. All determinations were done in triplicates.

(II) Influence of Nutrient Media on Cd Sorbed by Soil Particles in Sterile Conditions (Experiment II)

Sterile, autoclaved solution of $CdCl_2$ was added to the soil, autoclaved three times (the third time with the phosphate buffer - Bu) at 121°C for 30 min., after 24 hours intervals. Final concentrations of the soil and Cd in the phosphate buffer (Bu) were the same as in experiment I. After 24 hours of preincubation on a rotary shaker, autoclaved at 121°C for 15 min solutions of the nutrients (as in experiment I) or distilled water were added. All procedures were performed in the same way as in experiment I.

(III) Effect of Different Concentrations of the Phosphate Buffer on the Fate of Cd (Experiment III)

To obtain different buffer capacities of the soil suspensions, protecting the soil suspensions from pH changes, different concentrations of Bu were used. The soil samples (2.5 g) were suspended in 50 cm^3 of Bu at four different concentrations (0.036 M, 0.073 M, 0.182 M and 0.364 M). All suspensions contained 10 μg cm^{-3} Cd as $CdCl_2$. The soil suspensions were incubated with Cd at 30˚C for 24 hours, and afterwards either 2 cm^3 of Gl + mineral salts (see Experiment I, series 2) or Nb were added to make their final concentrations of 1% (w/v). After 1 or 3 days of incubation at 30°C on a rotary shaker, samples for Cd extraction as well as for determination of dehydrogenase activity and pH were taken. The Cd extraction and all determinations were performed as described by Bollag and Czaban [6]. Using the results of Experiment III, with Cd concentration in all examined fractions, the control values (of the soil suspensions in Bu) were subtracted, to clearly show the changes of Cd desorption or its extractability under microbial action. The results establish a "mirror reflection" picture between the changes of solid phase Cd extractability with NaOH and DTPA, and present a definitive relationship between Cd extractable with NaOH and Cd liberated to solution.

Statistical Evaluations

For estimation of all examined relationships simple correlation analysis was used. Together with correlation coefficients (r), probability (P) and the values number (n) are presented. For evaluation of relationship between changes of Cd extractability with NaOH and changes of Cd extractability with DTPA regression (polynomial and linear) analysis was applied. Together with the chosen equation of linear regression, determination coefficient (R^2), probability (P) and the examined values number (n) are presented. Statistical calculations were performed on results from which the control values of the soil suspensions in the corresponding buffer were subtracted.

Results

Changes of pH and Activity of Dehydrogenase

In sterile conditions, the addition of the nutrient media did not have a significant influence on the pH of soil suspensions. In these experimental series no dehydrogenase activity was detected (results not presented).

In non-sterile conditions, microbial transformation of both carbohydrates caused acidification of the soil suspension, with a maximum on the 2nd day of incubation. At that time, the pH of the nutrient media dropped from 7.3 at the beginning of the experiment, to 5.4 and 6.7 in the cases of Gl and St media, respectively. While on the contrary, microbial growth in Nb and PY increased the pH, with a maximum (pH = 9.0) on 4th and 5th days, respectively. In the case of soil suspension in Bu in non-aseptic conditions, the pH was not changing significantly during the examined period (Fig. 1A).

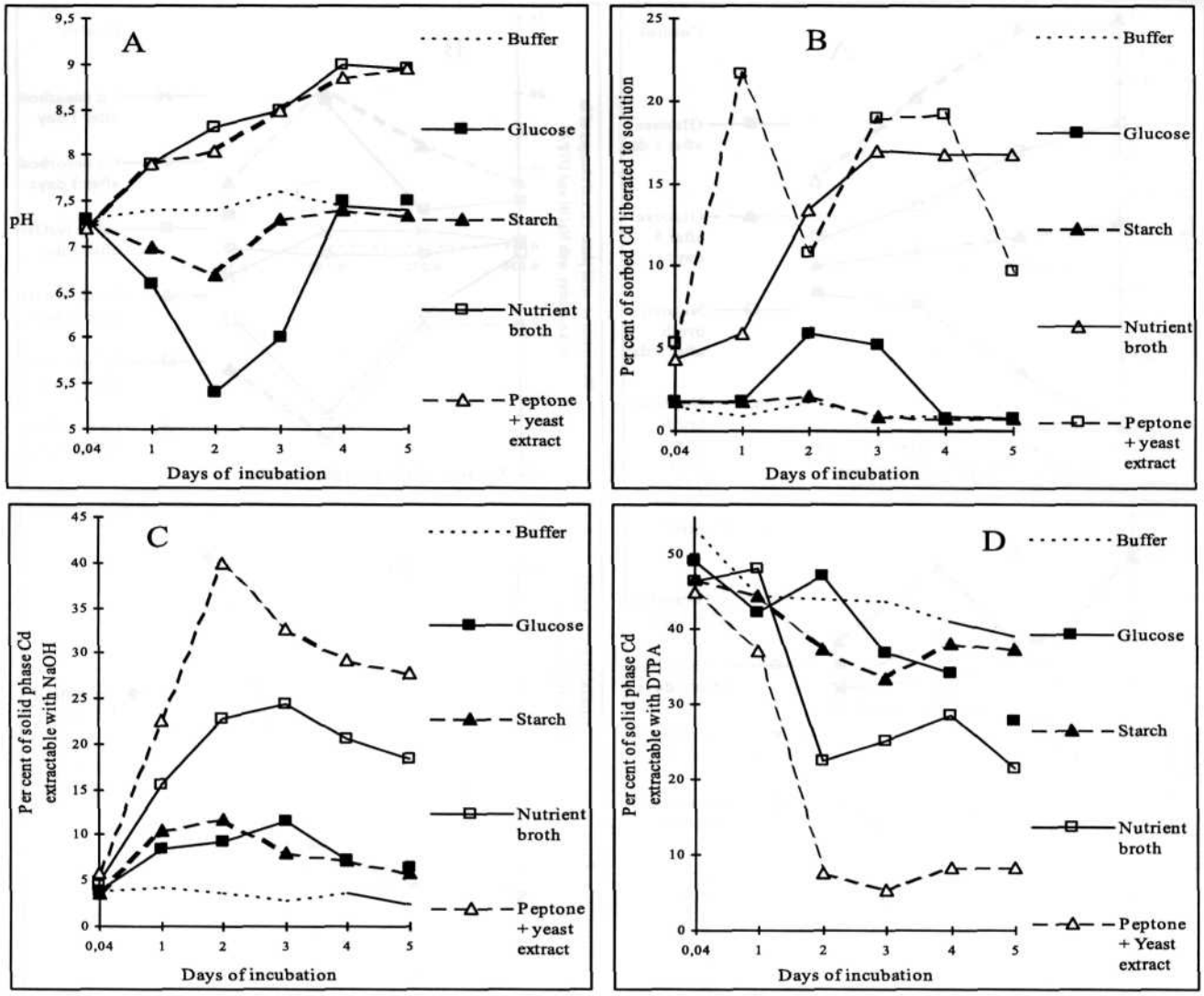

Fig. 1. Effect of microbial growth on pH changes and transformation of Cd sorbed by soil in soil suspension enriched with various nutrient media in Experiment I during 5 days of incubation at 30°C. (A) Changes of pH. (B) Liberation of solid phase Cd into liquid phase. (C) Changes of solid phase Cd extractability with NaOH solution. (D) Changes of solid phase Cd extractability with DTPA solution.

The changes of pH were accompanied by intensive dehydrogenase activity, indicating the abundant growth of microorganisms. The dehydrogenase activity in "the peptide media" was higher than in "the carbohydrate media" after 1 and 2 days, but when the pH in the series approached 9.0, after 4 and 5 days, the dehydrogenase activity dropped to zero (results not shown).

In experiment III, the increasing concentrations of the phosphate buffer (Bu) prevented, to some extent, the soil suspensions from pH changes (Fig. 2A).

As in experiment I, the intensive activity of dehydrogenase could be detected in the soil suspended in nutrient media (in this experiment especially after the addition of Gl), except there was no activity of dehydrogenase in the soil suspensions containing Nb dissolved in 0.036 M and 0.073 M Bu, after 3 days. The suspensions with Nb were very alkaline at the time (pH = 8.95 and 8.9). As in experiment I, at so high pH, no activity of dehydrogenase was detected (results not presented).

Sorption of Cd by Soil Particles

After 24 hr. preincubation of soil suspensions with $CdCl_2$, Cd was almost totally sorbed by the soil (more than 98% in sterile conditions - experiment Ib, and at least 97% in the other experiments).

Liberation (Desorption) of Sorbed Cd to the Liquid Phase

In experiments I and II, irrespective of the conditions (sterile or non-sterile), the concentration of Cd in liquid phase did not change significantly in all soil suspensions in Bu (the control series) and soil suspensions enriched with St.

The addition of Gl to the soil suspension in aseptic conditions did not influence the Cd desorption (results not presented), but in the non-sterile system, strong acidifica-

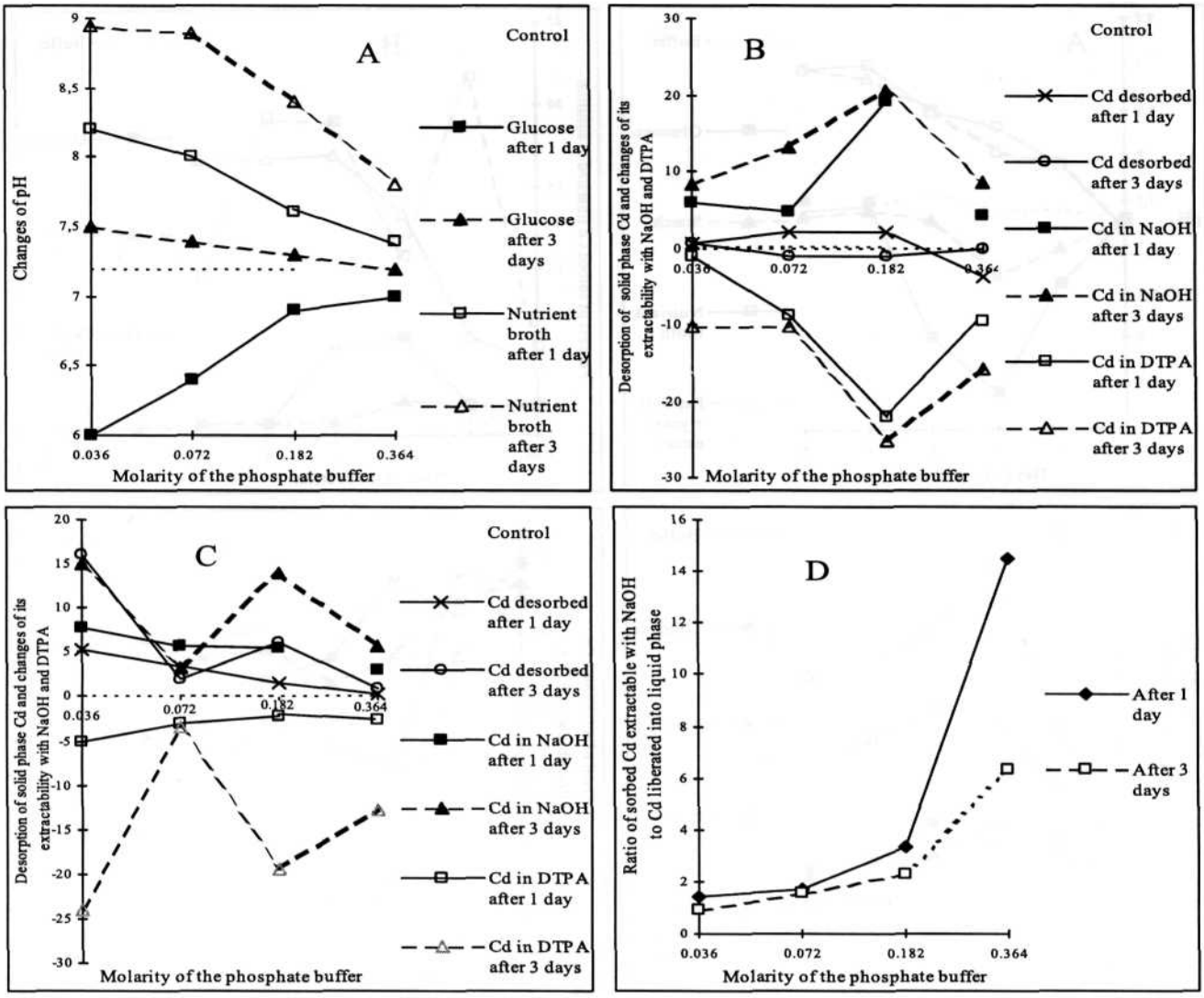

Fig. 2. Effect of microbial growth on pH changes and transformation of Cd sorbed by soil in soil suspension enriched with glucose or nutrient broth in Experiment III (with various concentrations of the phosphate buffer) after 1 or 3 days of incubation at 30°C. All results presented in figures B and C are obtained after subtracting the control (without nutrients added) values to show "a mirror reflection" of the changes of Cd extractability with NaOH and DTPA. (A) Changes of pH. (B) Liberation of solid phase Cd into liquid phase and changes of Cd extractability with NaOH and DTPA in soil suspension enriched with glucose. (C) Liberation of solid phase Cd into liquid phase and changes of Cd extractability with NaOH and DTPA in soil suspension enriched with nutrient broth. (D) Changes of ratio of solid phase Cd extractable with NaOH to solid phase Cd liberated into liquid phase in soil suspension enriched with nutrient broth.

tion due to Gl transformation to organic acids was accompanied by a 6% desorption of the sorbed Cd (Fig. IB).

In a sterile soil suspension system, Nb and PY liberated to solution about 4 and 5% of Cd sorbed by soil particles, respectively (results not presented). In non-sterile conditions, the liberation of Cd to the liquid phase was much more intensive during microbial utilization of PY (22%) and Nb (17% of sorbed Cd) (Fig. IB).

In experiment III, an increase of Bu concentration (without the nutrients) caused only a slight desorption of Cd. The percentage of sorbed Cd present in liquid phase in 0.036, 0.073, 0.182, and 0.364 M Bu amounted to respectively: 1,1, 2 and 5% on the 1st day, and 1, 2, 3 and 4% on the 3rd day of incubation (results not shown). As in Experiments I, the addition of Nb caused a much stronger liberation of Cd than Gl (results after subtracting the control values in Figs. 2B and 2C). In general, the increase of Bu concentration in the soil suspension amended with Nb protected Cd from liberation to the liquid phase (Fig. 2B).

Changes of Extractability of Solid Phase Cd with 0.1 M NaOH

In experiments I and II, after centrifugation of the soil suspension in Bu and separating the supernatants, the alkaline extractant removed 2-3% and 2.5-4.2% of sorbed Cd from the solid phase pellets obtained in sterile and non-sterile conditions, respectively (results not presented). Both "carbohydrate media" (Gl and St) did not

effect extractability of Cd with NaOH from separated (by centrifugation) solid phase pellets of the soil suspensions, incubated in sterile conditions. But, the "peptide media" (Nb and PY) increased these values by 3-6% (results not presented).

In non-sterile conditions the addition of nutrient media caused intensive microbial growth, as shown by dehydrogenase activity, after 2 or 3 days of incubation, what considerably increased the extractability of sorbed Cd with NaOH. After subtracting the control values (concerning soil suspension in Bu), the alkaline extractant could remove up to 8.8, 8.0, 21.7 and 36.3% from precipitates, obtained after centrifuging soil suspensions, enriched with Gl, St, Nb and PY (Fig 1C).

In experiment **III,** an increase of Bu concentration caused only a very slight decrease of Cd concentration in 0.1N NaOH extracts of the soil suspension pellets without nutrients added (results not presented). After adding the nutrient media a considerable increase of solid phase Cd concentration (extractable with NaOH) up to 20.7%, and 15.1% (after subtracting the control values) in the case of Gl and Nb, was respectively observed. There was no clear influence of the buffer concentration on Cd extractability with NaOH (Fig. 2B and 2C).

Ratio of Cd Extractable with 0.1 M NaOH to Cd Liberated to the Liquid Phase in Experiment HI

The lines, presented in Fig. 2C, concerning the changes of solid phase Cd liberated into liquid phase and of the remaining solid phase Cd extractability with NaOH in the soil suspensions enriched with Nb, show a similar pattern. But, the ratio $Cd_{NaOH}/Cd_{desorbed}$ gradually increased from 1.4 (through 1.7 and 3.4) to 14.5 on $1^{st,}$ day and from 0.9 (through 1.6 and 2.3) to 6.3 on 3^{rd} day of the incubation with the increase of Bu concentration from 0.036 (through 0.073 and 0.182) to 0.364 M (Fig. 2D).

Changes of Extractability of Solid Phase Cd with **DTPA**

In sterile conditions (Experiment II) DTPA removed 63-66% of Cd sorbed by soil suspended in Bu and 65-73%, 67-72%, 65-73% and 65-69% from soil suspended in Gl, St, Nb and PY media, respectively (results not shown).

In non-sterile conditions (Experiment I) after 1 hour of incubation, DTPA could extract 53.6, 49.1, 46.8, 46.4 and 44.9% of the sorbed Cd from pellets, obtained after centrifuging the soil suspensions in Bu and Gl, St, Nb and PY media, respectively. The extractability of solid phase Cd presented in Bu-suspension showed a tendency to decrease with the incubation time, down to 39.2% after 5 days (Fig. ID). The enrichment of soil suspensions with organic C media caused an additional strong decrease of Cd extractability with DTPA. The decrease of Cd extractability with DTPA (calculated after subtracting the control values) for individual series amounted to: -11.3, -10.2, -21.6 and -38.5% of solid phase Cd from soil suspensions containing Gl, St, Nb and PY, respectively (Fig. ID).

In experiment III, DTPA removed 51.6-58.5% of the solid phase Cd from an unenriched soil suspension after one day and 48.5-52% after 3 days. Similar to Experiment I, stimulation of microbial growth by the addition of nutrient media, caused a significant decrease of solid phase Cd extractability in DTPA (-25.3% of the solid phase Cd in the case of Gl, and -23.9% in the case of Nb) (Figs. 2B and 2C).

The Observed Relationships

When results of the control, concerning the extractability of Cd with DTPA and NaOH from suspensions in Bu, were subtracted from the nutrient media values, the "mirror reflection" was obtained. This is especially visible in Experiment **III** - with various Bu concentrations (Fig. 2B and 2C). During the intensive growth of soil microorganisms in soil suspensions of Experiment I, a significant, negative correlation was obtained between changes of sorbed Cd solubility in NaOH and DTPA ($r = -0.853$; $P < 0.01$; $n = 24$). In experiment **III,** the relationship was similar ($r = -0.866$; $P < 0.01$; $n = 16$). Significant (at $P < 0.01$) correlation coefficients concerning the relationship between changes of Cd extractability with NaOH and DTPA in the "carbohydrate media" and the "peptide media" amounted to -0.745 and -0.854, respectively (Table 1).

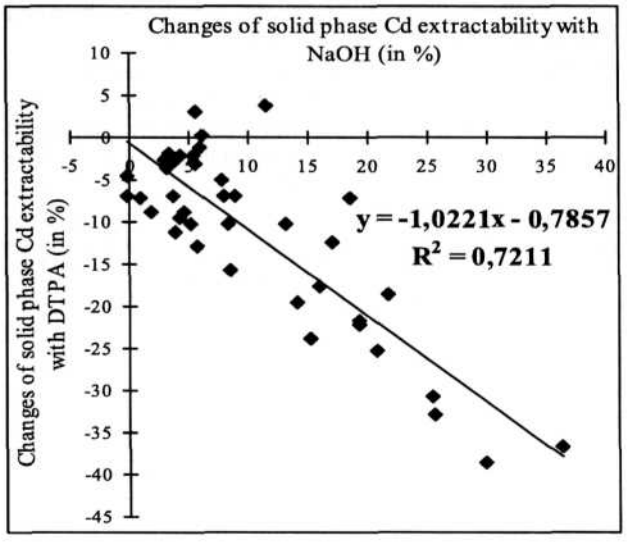

Fig. 3. Relationship between changes of extractability of Cd with NaOH and DTPA under the influence of microorganisms.

A following equation of linear regression (with $R^2 = 72.1\%$, $P = 0.0000$ and $n = 40$) describe the relationship between the changes of extractability of Cd with NaOH and DTPA (the results of both experiments and all media included):

$$y = -1.0221x - 0.7857;$$

where $y = Cd_{DTPA}$ and $x = Cd_{NaOH}$ (see Fig. 3).

Table 1. Correlation coefficients (r) between pH changes, changes of sorbed-Cd content liberated into liquid phase and changes of solid phase-Cd content extractable with NaOH and DTP A.

Nutrient medium	pH	Cd – desorbed (% of sorbed Cd)	Cd – NaOH (% of solid phase Cd)	Cd – DTPA (% of solid phase Cd)
Carbohydrates	1	–0.721***	0.067	–0.466**
		1	0.029	0.276
			1	–0.745***
Peptides	1	0.449**	0.385*	–0.399*
		1	0.714***	–0.579***
			1	–0.854***

***,**,* significant at $P < 0.001$, $P < 0.05$ and $P < 0.1$, respectively; ($n = 20$)

In the case of the "peptide media" (but not the "carbohydrate media") the significant correlation:

(1) positive ($r = 0.714$ at $P < 0.01$ with $n = 20$) between changes of Cd liberation to the liquid phase (after subtrac tion of the control values) and changes of extractability of sorbed Cd with NaOH (after subtraction of the control values) and

(2) negative ($r = -0.579$ at $P < 0.01$ with $n = 20$) be tween changes of Cd liberation to the liquid phase and changes of extractability of sorbed Cd with DTPA were observed (Table 1).

Both in the case of the "carbohydrate" ($r = -0.721$; $n = 20$; $P = 0.01$) and the "peptide media" ($r = 0.449$; $n = 20$; $P = 0.05$) a significant correlation between changes of pH and changes of Cd liberation to solution was obtained, but the relations were completely opposite (Table 1). The changes of pH during the microbial transformations of the all media were negatively correlated with changes of sorbed Cd extractability with DTPA ($r = -0.466$, $P < 0.05$, $n = 20$ and -0.399, $P < 0.1$, $n = 20$ in the case of the "carbohydrate" and "peptide media", respectively). The correlation (very weak) between pH changes and extractability with NaOH were observed only in the soil suspensions in the "peptide media" ($r = 0.385$ at $P < 0.1$; $n = 20$).

Discussion

The influence of abundant microbial growth on Cd sorbed by soil particles in soil suspension enriched with various nutrient media was the aim of this research. The nutrient media in sterile soil suspension system did not influence the fate of Cd greatly. Although both "peptide media" themselves could cause, in sterile conditions, a slight desorption of Cd and a slight increase of Cd extractability in 0.1 M NaOH (probably due to complexation of the heavy metal, as shown by Ramamoorthy and Kushner [29], and to sorption of these complexes on the soil particles), the main changes of Cd occurred undoubtedly as results of microbial growth, when high dehydrogenase activity was detected. The lack of dehydrogenase activities noted at the pH close to 9 could be the result of a negative effect of high ammonium concentration on the enzymes **[11].**

The changes of pH, through microbial transformations of Gl (acidification) and Nb and PY (alkalization), were connected with desorption of Cd bound to the soil particles. The former reaction was caused by a production of organic acids [32] but the latter could have at least two reasons. One is ammonification (Cd-desorbed in the "peptide media" was positively correlated with pH) and the other is a possible synthesis of low molecular organic products of peptide decomposition. All these compounds through complexation can easily transfer Cd to the liquid phase [4]. Another explanation of the phenomenon could be the possible solubilization of some microbial cell organic components, as was observed by Ledin *et al.* [25] at pH higher than 7. The fact that Cd liberated to the liquid phase in the "peptide media" was strongly positively correlated with fraction of Cd, extractable with NaOH, is proof of this suggestion. These changes of Cd content in NaOH extract should be proportional to the changes of microbial biomass, as shown by Bollag and Czaban [6]. It is confirmed that the ratio of Cd transformed to soluble in NaOH to Cd liberated to solution gradually increased with the increasing protective ability of the phosphate buffer.

The intensive growth of soil microflora in the suspensions with added nutrient media caused a significant transformation of Cd bound with the solid phase to forms soluble in 0.1N NaOH with simultaneous decrease of Cd extractability with DTPA. The changes showed almost a "mirror reflection" ($Cd_{NaOH} \approx -Cd_{DTPA}$). Similar changes of Cd extractability with NaOH and DTPA were noted by Bollag and Czaban [6] in soil suspension amended with sucrose and by Gambrell *et al.* (1976) - cited by Badura [1] in sediments of the Mississippi River. Bollag and Czaban [6] found that the increase of Cd solubility in NaOH is related to the biomass of microorganisms. Results of Kurek *et al.* [17] suggest that sorption by both bacteria and fungi could be responsible for the changes of Cd extractability with NaOH and DTPA.

Langley and Beveridge [22] conclude that phosphoryl groups were important sites involved in metal binding in *Bacillus* (teichoic and teichuronic acids), *Escherichia coli* (phospholipids and lipopolisaccharide) and *Pseudomonas aeruginosa* (lipopolisaccharide). Beveridge and Murray [3] and Beveridge *et al.* [2] found, that Gram positive bacteria growing in the presence of phosphate produce walls consisting primarily of teichoic acid and peptidoglycan. They found that extraction of the bacterial walls with 0.1 N NaOH removed teichoic acid, which substantially

reduced the ability of the walls to bind metals. Kurek *et al.* [18] showed that an extracellular protein from *Arthrobacter* sp. spent medium formed a water insoluble precipitate with Cd. It seems that precipitation of Cd by microorganisms as insoluble sulfides, carbonates or phosphates is also possible [5, 36]. It is very probable that binding to phosphoryl groups (e.g. of teichoic acid) on microbial cells is responsible for the changes of Cd extractability with the NaOH and DTPA solutions in the experiments presented in this paper. Some kinds of other precipitates (formed on microbial cells or out of them with their exudates) soluble in NaOH but not in DTPA, could be another explanation of the observed phenomenon. The uptake of Cd into microbial cells should also be taken into consideration.

As written previously [6], the inability of DTPA to extract Cd soluble in NaOH suggests that this form of Cd, bound to microbial cells or its exudates, is not available to plants [7, 12, 15, 21, 33, 34]. Results presented in many papers concerning sorption of Cd by microorganisms [14, 16, 17, 19, 23, 24, 25, 26, 28], also suggest that an accumulation of Cd by microorganisms is important for its immobilization in the natural environment. Recently, Kurek and Majewska [20] found that tops of lettuce, grown in soil supplemented with Cd bound to resting bacterial cells, contain significantly less Cd than from soil amended with CdCl2 or with Cd immobilized by clay or humic acids. On the other hand, various microbial activities (e.g. production of extracellular, water-soluble ligands and decomposition of soil organic materials containing Cd) may enhance the mobility of initially immobilized Cd [9, 10].

It seems, as in the soil suspension laboratory experiments presented in this paper, that in natural soils, especially in the rhizosphere - the natural environment of nutrients (and pollutants) taken up by plants, mobilization of Cd can occur simultaneously to its immobilization under microbial influence. It is hard to know whether the results of these microbial processes decreases or increases the availability of Cd to plants. Therefore, due to increasing pollution of the agricultural environment by heavy metals including Cd as one of the most dangerous elements, thorough studies on the heavy metals fate in the rhizosphere region with the simultaneous study on their accumulation by plants are needed.

Conclusions

1. Microbial transformation of carbohydrates to or ganic acids and peptides to ammonia caused partial mobil ization of Cd, initially sorbed by soil particles. Mobiliz ation of solid phase Cd in soil suspended in peptide media was probably also connected with dissolving of some part of microbial cells in pH higher than 7;

2. Simultaneously to this Cd mobilization, a transform ation of another fraction of Cd, connected with the solid phase, to form soluble in NaOH but not in DTPA, occur red. The sorption of Cd by microbial cells is the most likely explanation of this phenomenon;

3. All the observed transformations of Cd suggest that microbial action, especially in the rhizosphere region, can alter (both increase and simultaneously decrease) Cd availability to plants.

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