*Polish Journal of Environmental Studies Vol. 12, No. 4 (2003), 473-479*

# Effect of Cadmium and Magnesium on Enzymatic Activity in Soil

**J. Wyszkowska*, M. Wyszkowski**

University of Warmia and Mazury in Olsztyn, Department of Microbiology, Plac Lódzki 3, 10-727 Olsztyn, Poland

*Received: 9 January, 2003*
*Accepted: 7 February, 2003*

**Abstract**

The purpose of this study was to use magnesium (50 and 100 mg Mg $kg^{-1}$) to neutralise possible negative effects of soil contamination with cadmium (10, 20, 30 and 40 mg Cd $kg^{-1}$) on the enzymatic activity of soil.

Soil contamination with cadmium depressed the activity of dehydrogenases, urease, alkaline phosphatase and, to a smaller extent, acid phosphatase. In addition, cadmium pollution narrowed down the potential biochemical fertility index of soil under yellow lupine. Application of magnesium to soil alleviated the unfavourable impact of cadmium on dehydrogenases during the shoot elongation phase. Such positive influence of magnesium on urease was observed both during the shoot elongation phase and at harvest, and in the case of alkaline and acid phosphatases it occurred only at harvest.

**Keywords:** cadmium contamination, magnesium fertilisation, soil enzymes, yellow lupine yield.

## Introduction

By taking part and playing an important role in chemical changes of carbon, nitrogen, phosphorus and sulphur compounds [1], soil enzymes can serve as a tool to determine biochemical soil properties. For this purpose, activity of dehydrogenases is most commonly assayed, as it is usually positively correlated with the volume of yields, which in turn may indicate, however indirectly, that the activity of those enzymes is related to soil fertility [2]. Activity of other soil enzymes, such as urease or phosphatase, can also be helpful because both enzymes are as sensitive as dehydrogenases in indicating processes occurring in soil.

Contamination with heavy metals, fertilisation, application of plant protection chemicals and soil cultivation all modify physicochemical characteristics of soil and change its biological activity [3]. Of special importance are heavy metals, which may stimulate the activity of soil enzymes if present in small amounts, but will act as inhibitors if found in high concentrations [4, 5, 6, 7]. The effect of heavy metals on biological activity of soil depends on the physicochemical properties of soil, particularly on its humic content. On the other hand, it is also dependent on concentrations as well as kinds of pollutants or enzymes involved [4, 5, 8]. Cadmium represents a group of heavy metals causing the most severe changes in the biological properties of soils [9, 10, 11, 12, 13].

The purpose of our study was to use magnesium to neutralise possible negative effect of cadmium contamination of soil on its enzymatic activity as well as to determine the relationship between the enzymes and yields of yellow lupine or some physical and chemical characteristics of soil.

## Material and Methods

Yellow lupine cv. Juno was grown in a pot experiment established in a greenhouse at the University of Warmia and Mazury in Olsztyn (north-eastern Poland). Simulated soil contamination was performed using 0, 10, 20, 30 and 40 mg Cd $kg^{-1}$ of soil. The tests were carried out in three replications with increasing rates of magnesium: 0, 50 and 100 mg Mg $kg^{-1}$ of soil, against the background of

*Corresponding author, e-mail: jadwysz@uwm.edu.pl

constant fertilisation with other macroelements: nitrogen (25 mg N), phosphorus (33 mg P) and potassium (75 mg K $kg^{-1}$ of soil). The experiment was carried out in plastic pots filled with 10 kg of soil collected from $A_p$ humic layer of soil from farmland. Under natural conditions, the soil had the following characteristics: soil size fraction of heavy loamy sand, $pH_{KCl}$ – 5.0; hydrolytic acidity (H) – 22.5 mmol $H^+ kg^{-1}$; total exchange bases (S) – 61.0 mmol $H^+ kg^{-1}$; total exchange capacity (T) – 83.5 mmol $H^+ kg^{-1}$; degree of base saturation (V) – 73.1%; P content – 99.2 mg; K content – 110.3 mg and Mg content – 9.1 mg $kg^{-1}$ of soil.

Cadmium as applied in the form of $CdCl_2$, nitrogen as $CO(NH_2)_2$, phosphorus as $KH_2PO_4$, potassium as $KH_2PO_4$+KCl and magnesium as $MgSO_4 \cdot 7H_2O$. Cadmium, nitrogen, phosphorus, potassium and magnesium compounds were dissolved in water and applied once by thoroughly mixing the solutions with soil when establishing the experiments.

Samples of soil were taken during the vegetative growth of yellow lupine: at shoot elongation phase (1st date) and at harvest (2nd date), to determine the activity of some soil enzymes: dehydrogenases (Deh) by Lenhard's methods modified by Casida et al. [14], urease (Ure) by the methods of Gorin and Ching Chang [15], and acid phosphatase (Pac) and alkaline phosphatase (Pal) according to Tabatabai and Bremner [16]. The activity of those enzymes was determined in the colorimeter by measuring the value of extinction on the spectrometer at a wavelength of 410 nm (urease, acid and alkaline phosphatases) and 485 nm (dehydrogenases).

Soil was also analyzed for organic carbon content (%C), but due to the fact that simulated cadmium contamination and magnesium fertilisation did not produce any significant changes in the soil under yellow lupine, those results were not included in the paper and average carbon content was 1.02%. On the basis of enzymatic activity and carbon content, potential biochemical soil fertility index was computed from the formula [17]:

$$M_W = (Ure\ 10^{-1} + Deh + Pal + Pac)\ \ \%C$$

Statistical interpretation of the results involved two- and three-factor analysis of variance. Significance of the rates of the factors applied was estimated at significance levels of p=0.05 and p=0.01. The relationships between the activity of soil enzymes and cadmium rates of yields of aboveground parts and roots of lupine, as well as the number and weight of root nodules, were determined using polynominal regression equations, while those between cadmium contamination and magnesium rates versus chemical and biochemical soil properties were assessed using Pearson's simple correlation coefficients. All statistical calculations were performed using the Statistica software package [18].

## Results and Discussion

Soil contamination with cadmium had a strong, antagonistic effect on the activity of soil enzymes. The least tolerant to the contamination were dehydrogenases. Under the influence of the highest rate of cadmium, in the non-fertilized soil analyzed at shoot elongation phase their activity was depressed by 61% (r=-0.86) compared to the uncontaminated soil (Table 1). Later, as the vegetative growth of yellow lupine continued, the effect of cadmium weakened and the activity of dehydrogenases in soil increased. In the control series of experiments (without Mg), the activity of those enzymes after the yellow lupine harvest was on average c. 94% higher than on the first date of analyses. After harvest, decrease in the activity of dehydrogenases caused by the toxic effect of cadmium was smaller than at shoot elongation phase, reaching 46% (r=-0.91). Application of magnesium to soil alleviated the significant negative effect of cadmium on dehydrogenases at shoot elongation phase, so that their activity was depressed to 55% (r=-0.97) in the series with 100 mg Mg $kg^{-1}$ of soil. After harvest, such favourable effect of magnesium on the activity of dehydrogenases was obtained only by the application of 50 mg Mg $kg^{-1}$ of soil. Toxic influence of cadmium on the activity of dehydrogenases was reported by other authors, e.g. Milosevic et al. [9], Olszowska [19] and Welp [12]. Soil contamination with cadmium can depress the activity of those enzymes by up to 80-95% relative to the control [19]. In the research by Kandeler et al. [20], cadmium concentration as low as 3 mg $kg^{-1}$ caused a 54-69% reduction of the activity of dehydrogenases due to a joint effect of cadmium and other heavy metals (zinc, copper, nickel and vanadium). Welp [12] claimed that heavy metals reduced the activity of dehydrogenases by 10 to 90% depending on the rate and type of metals. In the latter study, the toxic effect of heavy metals on the activity of dehydrogenases, expressed as the rate of a heavy metal responsible for a 50% decrease in enzymatic activity, declined in the following order: Hg (2mg)>Cu (35mg)>$Cr_{(VI)}$ (71mg)> $Cr_{(III)}$ (75mg)>Cd (90mg)>Ni (100mg)>Zn (115mg)>As (168mg)>Co (582mg)>Pb (652mg $kg^{-1}$ of soil). Sorption of metals and their microbiological toxicity revealed certain unique characteristics: larger amounts of metals were sorbed by soil at higher toxicity of those metals in the soil environment.

Activity of urease showed some analogy to that of dehydrogenases, i.e. it decreased significantly at higher rates of cadmium (Table 1). This observation is consistent with the reports by Landi et al. [10], Lebedeva et al. [11] and Zheng-ChunRong et al. [13]. However, the decrease in the activity of urease was smaller compared to dehydrogenases, e.g. in the series without magnesium the activity of urease was 26% lower both during the vegetative growth and after the harvest of yellow lupine. Application of magnesium to soil had a limiting effect on the decrease of the activity of urease in the cadmium-contaminated objects, with the lower magnesium rate producing a more favourable effect on the first date of analyses and

Table 1. Enzymatic activity in 1 kg d.m. of soil.

| Cd dose in mg $kg^{-1}$ of soil | Dehydrogenases ($cm^3$ $H_2$ $d^{-1}$) | | Urease (mg N-$NH_4$ $h^{-1}$) | | Acid phosphatase (mmol PNP $h^{-1}$) | | Alkaline phosphatase (mmol PNP $h^{-1}$) | | $M_w$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1st date | 2nd date | 1st date | 2nd date | 1st date | 2nd date | 1st date | 2nd date | 1st date | 2nd date |
| Without Mg | | | | | | | | | | |
| 0 | 1.50 | 3.11 | 7.23 | 6.21 | 2.58 | 3.68 | 1.11 | 0.76 | 11.62 | 10.77 |
| 10 | 1.46 | 2.46 | 6.09 | 5.64 | 2.74 | 3.49 | 0.97 | 0.58 | 10.05 | 10.09 |
| 20 | 1.45 | 2.41 | 5.77 | 5.51 | 2.81 | 2.55 | 0.81 | 0.59 | 9.52 | 9.13 |
| 30 | 1.19 | 2.41 | 5.70 | 4.85 | 2.58 | 2.42 | 0.84 | 0.58 | 9.27 | 8.63 |
| 40 | 0.58 | 1.69 | 5.35 | 4.59 | 2.56 | 2.63 | 0.80 | 0.58 | 9.46 | 8.50 |
| Average | 1.24 | 2.41 | 6.03 | 5.36 | 2.65 | 2.96 | 0.91 | 0.62 | 9.98 | 9.42 |
| r | -0.86 | -0.91 | -0.91 | -0.98 | -0.27 | -0.86 | -0.90 | -0.70 | -0.84 | -0.97 |
| 50 mg Mg $kg^{-1}$ of soil | | | | | | | | | | |
| 0 | 1.55 | 3.30 | 6.27 | 6.14 | 2.78 | 2.71 | 1.13 | 0.83 | 10.08 | 9.83 |
| 10 | 1.44 | 2.82 | 5.97 | 6.04 | 2.77 | 2.86 | 0.96 | 0.68 | 10.33 | 9.39 |
| 20 | 1.34 | 2.78 | 5.74 | 5.80 | 2.66 | 2.78 | 0.82 | 0.59 | 9.68 | 9.14 |
| 30 | 1.16 | 2.32 | 5.81 | 5.32 | 2.65 | 2.82 | 0.81 | 0.57 | 9.71 | 8.65 |
| 40 | 0.66 | 1.90 | 5.18 | 4.87 | 2.65 | 2.77 | 0.66 | 0.57 | 8.86 | 8.25 |
| Average | 1.23 | 2.62 | 5.79 | 5.63 | 2.70 | 2.79 | 0.88 | 0.65 | 9.73 | 9.05 |
| r | -0.93 | -0.98 | -0.93 | -0.97 | -0.90 | 0.23 | -0.97 | -0.90 | -0.87 | -1.00 |
| 100 mg Mg $kg^{-1}$ of soil | | | | | | | | | | |
| 0 | 1.66 | 3.67 | 6.34 | 6.94 | 2.66 | 3.05 | 1.22 | 0.84 | 11.07 | 11.02 |
| 10 | 1.51 | 2.51 | 5.84 | 6.39 | 2.86 | 3.06 | 1.00 | 0.79 | 10.70 | 9.75 |
| 20 | 1.40 | 2.34 | 5.53 | 6.21 | 2.64 | 2.78 | 0.84 | 0.80 | 10.14 | 10.21 |
| 30 | 0.88 | 2.40 | 5.40 | 6.23 | 2.60 | 2.84 | 0.82 | 0.80 | 9.43 | 10.54 |
| 40 | 0.74 | 1.68 | 4.96 | 5.75 | 2.61 | 2.34 | 0.79 | 0.75 | 8.44 | 8.58 |
| Average | 1.24 | 2.52 | 5.61 | 6.30 | 2.67 | 2.81 | 0.93 | 0.79 | 9.96 | 10.02 |
| r | -0.97 | -0.90 | -0.98 | -0.94 | -0.51 | -0.89 | -0.91 | -0.85 | -0.98 | -0.70 |
| LSD | a – 0.07**; b – 0.05**; c – 0.04**; axb – 0.12**; axc – 0.10**; bxc – 0.08**; axbxc – 0.17** | | a – 0.31**; b – 0.25*; c – n.s.; axb – n.s.; axc – n.s.; bxc – 0.35**; axbxc – n.s. | | a – 0.08**; b – 0.06*; c – 0.05**; axb – 0.13**; axc – 0.11**; bxc – 0.08**; axbxc – 0.19** | | a – 0,02**; b – 0,02**; c – 0,02**; axb – 0,04**; axc – 0,03**; bxc – 0,03**; axbxc – 0,06** | | a – 0.35**; b – 0.27**; c – 0.23**; axb – 0.13**; axc – n.s.; bxc – 0.40**; axbxc – 0.88* | |

$M_w$ - potential biochemical index of soil fertility, LSD for: a – Cd dose, b – Mg dose, c – for date of analysis; n.s. – non-significant, * significant at p=0.05, ** significant at p=0.01

the higher one – after the harvest. In their experiments, Park-Hyun et al. [21] found that mineral fertilisation, including magnesium treatments, led to lowered activity of dehydrogenases.

Of all the soil enzymes assayed in our tests, acid phosphatase was the least affected by cadmium contamination (Table 1). Cadmium contamination caused only some small fluctuations in the activity of acid phsophatase at the shoot elongation phase. However, after harvest, it was found to be responsible for a significantly negative effect on the activity of this enzyme, especially in the series without magnesium, which was confirmed by the correlation coefficient computed as r=-0.86. Such strong influence of cadmium contamination of soil on the activity of acid phosphatase was also reported by Nowak et al. [3], who determined that it could decrease by 15-25% (15 mg

Table 2. Polynominal regression equations between the rates of Cd and activity of soil enzymes in 1 kg d.m. of soil or the potential biochemical index of soil fertility, irrespective of the magnesium fertilisation and date of soil analysis.

| | Polynominal regression equations | $R^2$ |
|---|---|---|
| Dehydrogenases | $y = -0.0002x^2 - 0.0189x + 2.3903$ | 0.948 |
| Urease | $y = 0.0002x^2 - 0.0387x + 6.4674$ | 0.975 |
| Acid phosphatase | $y = -7E-06x^2 - 0.0092x + 2.9506$ | 0.843 |
| Alkaline phosphatase | $y = 0.0002x^2 - 0.015x + 0.9714$ | 0.969 |
| Potential biochemical index of soil fertility | $y = -0.0014x^2 - 0.0126x + 10.578$ | 0.938 |

Table 3. Pearson's simple correlation coefficients between the rates of Cd and Mg, yellow lupine yield, some chemical and biochemical soil properties, irrespective of the date of analysis (mean for all series with magnesium).

| | Deh | Ure | Pac | Pal | $M_w$ |
|---|---|---|---|---|---|
| Cd dose | -0.51** | -0.74** | -0.49** | -0.56** | -0.74** |
| Mg dose | 0.03 | 0.17 | -0.09 | 0.25* | 0.13 |
| aboveground parts | 0.45** | 0.66** | 0.50** | 0.44** | 0.64** |
| weight of roots | 0.48** | 0.69** | 0.39** | 0.50** | 0.63** |
| number of nodules | 0.39** | 0.43** | 0.29** | 0.23* | 0.40** |
| mass of nodules | 0.48** | 0.67** | 0.39** | 0.48** | 0.60** |
| $pH_{KCl}$ | 0.79** | 0.22* | 0.45** | -0.39** | 0.01 |
| H | -0.81** | -0.05 | -0.41** | 0.61** | 0.12 |
| S | 0.56** | 0.53** | 0.40** | 0.24* | 0.50** |
| T | -0.38** | 0.30** | -0.11 | 0.72** | 0.44** |
| V | 0.89** | 0.19 | 0.48** | -0.49** | 0.02 |
| C | -0.41** | -0.22* | -0.26* | 0.22* | 0.33** |
| P | -0.34** | -0.04 | -0.14 | 0.27** | 0.05 |
| K | -0.64** | -0.03 | -0.31** | 0.49** | 0.09 |
| Mg | -0.04 | 0.21* | -0.09 | 0.36** | 0.22* |
| Deh | 1.00 | 0.35** | 0.51** | -0.28** | 0.20 |
| Ure | 0.35** | 1.00 | 0.27** | 0.59** | 0.80** |
| Pac | 0.51** | 0.27** | 1.00 | -0.11 | 0.37** |
| Pal | -0.28** | 0.59** | -0.11 | 1.00 | 0.67** |
| $M_W$ | 0.20 | 0.80** | 0.37** | 0.67** | 1.00 |

H – hydrolytic acidity; S – total exchange bases; T - sorptive capacity; V - degree of base saturation; C, P, K, Mg – C, P, K, Mg content in soil; Deh - dehydrogenases, Ure - urease, Pac - acid phosphatases, Pal - alkaline phosphatases, Mw - potential biochemical index of soil fertility. Correlation coefficients for n=90.

Cd kg$^{-1}$) to 20-30% (75 mg Cd kg$^{-1}$). Depressed activity of acid phospatase in soil with high concentrations of cadmium was also revealed in studies conducted by Landi et al. [10]. According to Zheng-ChunRong et al. [13] heavy metals, including cadmium, produce an inactivating effect on acid phosphatase. Application of magnesium at 50 mg Mg kg$^{-1}$, contrary to higher magnesium rates, alleviates the negative effect of Cd on acid phsophatase. Although cadmium did have an adverse effect of acid phosphatase in the series treated with 100 mg Mg kg$^{-1}$, it was less severe than in the magnesium unfertilised objects. Park-Hyun et al. [21], however, suggested that the activity of

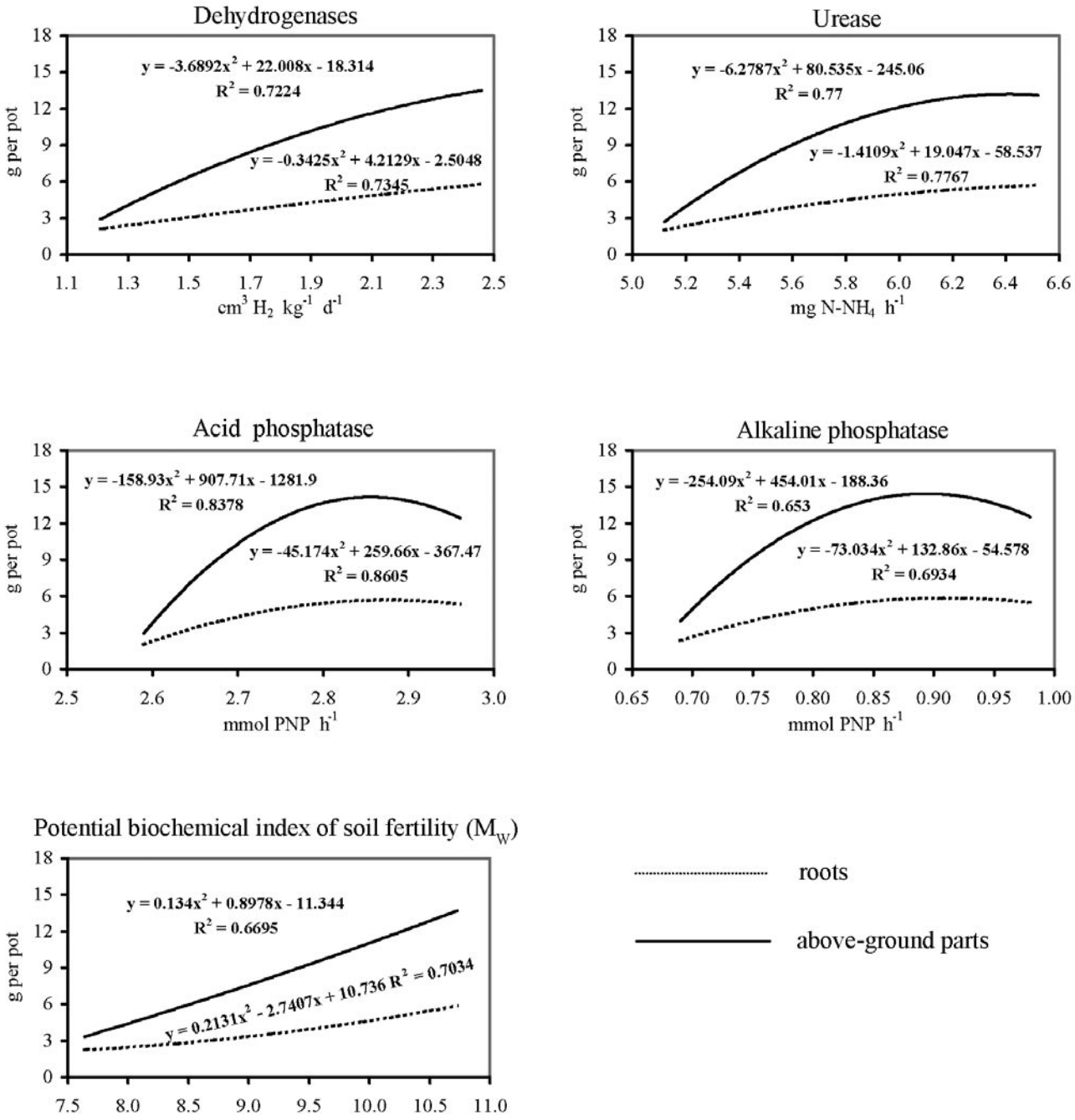


Fig.1. Relationships between the activity of enzymes in 1 kg d.m. of soil and the potential biochemical index of soil fertility ($M_w$) and the yield of above-ground parts and weight of roots of yellow lupine.

phosphatase did not necessarily depend on the application of magnesium. The activity of acid phosphatase was significantly higher at harvest than during the vegetative growth of yellow lupine.

The activity of alkaline phosphatase was negatively correlated with the cadmium contamination level found in soil (Table 1). Soil fertilisation with magnesium retarded the decrease in the activity of alkaline phosphatase (by 11%) at increasing rates of cadmium, but only in the series treated with 100 mg Mg kg$^{-1}$. Park-Hyun et al. [21], in contrast, did not detect any changes in the activity of phosphatase related to magnesium fertilisation. The highest drop in the activity of alkaline phosphatase caused by cadmium was observed in the series with 50 mg Mg kg$^{-1}$, reaching 42% at shoot elongation phase and 30% at the yellow lupine harvest. The respective correlation coefficients were r=-0.97 and r=-0.90. Of interest is the fact that the activity of alkaline phosphatase was considerably higher during the vegetative growth of yellow lupine than during its harvest. The decrease in the activity of alkaline phosphatase caused by cadmium was close to that determined by Nowak et al. [3], but much smaller than reported by Kandeler et al. [20].

The results of our experiments enable us to assume that levels of the activity of a single soil enzyme are sufficient to make predictions about soil fertility. A much more comprehensive view of the biochemical changes occurring in soil can be attained by calculating potential biochemical soil fertility index ($M_W$), which involves such components as the activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and the content of organic carbon [21]. In the present study, the potential biochemical index of soil fertility depended on the simulated contamination of soil with cadmium and, less strongly, on the date of analysis and rate of magnesium (Table 1). At higher rates of cadmium, the potential biochemical index of soil fertility became considerably narrowed. Noteworthy is the fact that the most extensive range of such changes appeared in the series treated with 100 mg Mg $kg^{-1}$ of soil (r=-0.97) and the smallest in the objects fertilised with 50 mg Mg $kg^{-1}$ of soil (r=-0.91).

Regardless of the rate of magnesium fertilisation or the date of soil analyses, strong negative correlation between the rate of cadmium and activity of enzymes as well as with the potential biochemical soil fertility index was confirmed by computing 2nd degree polynominal regression equations (Table 2). In their research, Dar [22] and Schuller [23] also suggested significant negative correlation between the concentration of cadmium in soil and activity of dehydrogenases and phosphatases. Soil enzymatic activity can be one of the factors which will allow us to predict the volume of yields. The experiments verify the occurrence of strong and significant correlation between the activity of dehydrogenases, urease and the potential biochemical soil fertility index as well as some weaker correlation between acid and alkaline phosphatases with the yield of aboveground parts and weight of roots or the weight of nodules on roots of yellow lupine. This is proved by polynominal regression equations (Fig. 1) and Pearson's simple correlation coefficients (Table 3). The activity of soil enzymes was correlated not only with the yield of plants but also with some chemical properties of soil, especially with the soil's reaction, hydrolytic acidity, and partly with sorptive properties. It was also correlated with the content of magnesium at the harvest of yellow lupine (Table 3). The activity of soil enzymes can therefore serve as an indicator for predicting crops yields.

## Conclusions

1. Soil contamination with cadmium depressed the activity of dehydrogenases, urease, alkaline phosphatase and, less strongly, acid phosphatase. It also resulted in the narrowing of the potential biochemical soil fertility index for soil under yellow lupine. Cadmium contamination had the strongest effect on dehydrogenases.
2. Antagonistic effect of cadmium on the activity of dehydrogenases and alkaline phosphatase, unlike acid phosphatase, was stronger at shoot elongation phase than at harvest.
3. Magnesium applied to soil alleviates the negative effect of cadmium on dehydrogenases at shoot elongation phase. It produces similar effects on urease at both terms of analyses and on acid and alkaline phosphatase at harvest.
4. The activity of dehydrogenases, urease and the value of the potential biochemical soil fertility index were significantly correlated with the yield of aboveground parts, weight of roots, weight of nodules on yellow lupine roots and with some physicochemical properties of soil. A similar, but weaker, correlation was observed for acid and alkaline phosphatases.

## References


1. DICK W.A. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. Soil Sci. Soc. Amm. J. **48**(3), 569, **1984**.
2. KOBUS J. Biologiczne procesy a kształtowanie żyzności gleby. Zesz. Probl. Post. Nauk Rol. **421a**, 209-219 (in Polish, with English abstract), **1995**.
3. NOWAK J., NIEDZWIECKI E., DZIEL M. Wpływ metali ciężkich na zmiany aktywności enzymatycznej gleby. Rocz. Gleboz. **50**(1/2), 61 (in Polish, with English abstract), **1999**.
4. CHRISTENSEN G.M., OLSON D. RIEDEL B. Chemical effects on the activity of eight enzymes. A review and a discussion revelant to environmental monitoring. Environ. Res. **29**, 247, **1982**.
5. FRANKENBERGER W.T.JR., JOHANSON J.B. AND NELSON C.O. Urease activity in sewage sludge-amended soils. Soil Biol. Biochem. **15**, 543, **1983**.
6. KUCHARSKI J., WYSZKOWSKA J. Microbiological properties of soil contaminated with chromium. Natural Sc. **7**, 7, **2000**.
7. WYSZKOWSKA J., KUCHARSKI J., JASTRZEBSKA E. AND HLASKO A. The biological properties of the soil as influenced by chromium contamination. Polish J. Environ. Stud. **10**(3), 175, **2001**.
8. MORENO J.L., GARCIA C., LANDI L., FALCHINI L., PIETRAMELLARA G., NANNIPIERI P. The ecological dose value ($ED_{50}$) for assessing Cd toxicity on ATP content and dehydrogenase and urease activities of soil. Soil Biol. Biochem. **33**(4-5), 483, **2001**.
9. MILOSEVIC N., GOVEDARICA M., JARAK M., PETROVIC N., JEVTIC S., LAZIC B. The effect of heavy metals on total soil microbiological activity in lettuce. Acta Horticul. **462**, 133, **1997**.
10. LANDI L., RENELLA G., MORENO J.L., FALCHINI L., NANNIPIERI P. Influence of cadmium on the metabolic quotient, L:-D-glutamic acid respiration ratio and enzyme activity:microbial biomass ratio under laboratory conditions. Biol. Fert. Soils. **32**(1), 8, **2000**.
11. LEBEDEVA L.A., LEBEDEV S.N., EDEMSKAYA N.L. The effect of heavy metals and lime on urease activity in soddy-podzolic soil. Moscow Univ. Soil Sci. Bull. **50**(2),53, **1995**.
12. WELP G. Inhibitory effects of the total water-soluble concentrations of nine different metals on the dehydrogenase activity of a loess soil. Biol. Fert. Soils, **30**(1-2), 132, **1999**.
13. ZHENG C.R., TU C., CHEN H.M. Effect of combined heavy metal pollution on nitrogen mineralization potential, urease and phosphatase activities in a typic udic ferrisol. Pedosphere. **9**(3), 251, **1999**.

14. CASIDA L.E, KLEIN J.D., SANTORO D. Soil dehydrogenases activity. Soil Sci. **98**, 371, **1964**.
15. GORIN G., CHING CHANG CH. A new method of assay the specific enzymic activity. IV Urease. Analyt. Biochem. **17**, 49, **1966**.
16. TABATABAI M.A., BREMNER J.M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. **1**, 307, **1969**.
17. KUCHARSKI J. Relacje między aktywnością enzymów a żyznością gleby. In: Drobnoustroje a życie gleby. AR Kraków (ed. W. Barabasz), 327 (in Polish, with English abstract), **1997**.
18. StatSoft, Inc. STATISTICA for Windows [Computer program manual]. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK 74104, http://www.statsoft.com. 2000.
19. OLSZOWSKA G. Effect of cadium-zinc dusts on the activity of various soil enzymes. Pr. Inst. Bad. Leśn. **847**, 111, **1998**.
20. KANDELER E., MENTLER A., PFEFFER M., HORAK O. Soil biological evaluation of heavy metal toxicity in artifically polluted soils. Verband Deutsch. Landwirtsch. Untersuch. Forschungsanst. **32**, 621, **1990**.
21. PARK H., YOO J.H., LEE C.H., BYUN J.K., KIM, Y.K. Effects of liming and fertilization on forest soil chemical properties, bacterial population and soil enzyme activities. FRI J. Forest Sci. Seoul. **58**, 178, **1998**.
22. DAR G.H. Effects of cadmium and sewage-sludge on soil microbial biomass and enzyme activities. Biores. Tech. **56**(2-3), 141, **1996**.
23. SCHULLER E. Enzyme activities and microbial biomass in old landfill soils with long-term metal pollution. Verhandlun. Gesellsch. Okol. **18**, 339, **1989**.