AKTIVITAS ENZIM FOSFATASE DI DALAM TANAH DENGAN PERLAKUAN LOGAM BERAT

THE ACTIVITY OF SOIL ACID-PHOSPHATASE AT ELEVATED CONCENTRATION OF HEAVY METALS

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Abstrak *Aktivitas enzim fosfatase tanah dilaporkan terpengaruh oleh logam berat, namun data tentang pengaruh setiap unsur logam berat terhadap aktivitas enzim tersebut, khususnya di tanah tropika, sangat jarang. Penelitian ini bertujuan untuk mengetahui pengaruh beberapa jenis unsur logam berat terhadap aktivitas fosfatase asam di dalam tanah tropika dan iklim sedang. Hasil percobaan menunjukkan bahwa aktivitas enzim ini menurun drastis akibat perlakuan Pb atau Cd, namun tidak terpengaruh oleh perlakuan Cu atau Fe. Penambahan Cd dengan takaran 40 mg* kg^{-1} *menurunkan aktivitas fosfatase asam sebesar 44% dalam sebuah tanah Jepang dan sebesar 43% dalam sebuah tanah Indonesia; sedangkan penambahan Pb dengan takaran sama menurunkan aktivitas fosfatase asam sebesar 54% dalam tanah Jepang dan sebesar 39% dalam tanah Indonesia.*

Kata kunci : *asam fosfatase, logam berat, timbal, kadminium, tembaga, besi.*

Abstract *The activities of phosphatases in soils have been suggested to be affected by heavy metals, but data on the effect of individual metal element, particularly in tropical soils, is scanty. This research evaluated the effect of some heavy metals the activity of acid phosphatase in tropical and temperate soils. The results showed that the activity of acid phosphatase was significantly reduced by either by Pb or Cd, but not by Cu or Fe. Addition of Cd at 40 mg* kg^{-1} *reduced the acid phosphatase activity as much as 44% in Japanese soil and 43% in an Indonesian soils; while Pb of the same level decreased the acid phosphatase activity as much as 54% in the Japanese soil and 39% in the Indonesian soil.*

Key words : *phosphatase acid, heavy metals, lead, cadminium, copper, iron.*

INTRODUCTION

The activities of phosphatase in soils have been suggested by many researchers to have an important role in providing available phosporus (P) to plants by accelerating the transformation of organic P to orthophosphate, an inorganic form of P that the available to plants [10]. Their activities have been shown to be related to the organic P and available form of P in soils and were affected by some environmental factors such as pH, temperature, organic-matter contents, water content, tillage practices, etc. Current trend of the increasing soil levels in heavy metal elements derived from the use of P-fertilizers, pesticides, and/or industrial wastes in agricultural land has also been suggested to influence phosphatase activities.

Several worker have reported some relationships between heavy metal concentrations and soil phosphatase activities in soil; most suggested that the activities of phosphatase in soils may be decreased by elevating heavy-metals concentrations [4,6,9,11]. Juma and Tabaitabai (1977) showed that Cu, Fe, Pb, Cd, Ni, and Zn were among the heavy metals that inhibit the soil acid phosphatase, which by an addition of 25 µmole metal per g soil decreased the enzyme activity as much as 18.7 to 47.7 %. The magnitude of the metal addition in their experimental units was high, but it clearly demonstrated the negative effect of heavy metal on phosphatase activities in

heavily polluted soils. Mathur et al. (1980) [5] also showed that the phosphatase and microbial activities were lower ini Histosols with higher concentrations of Cu than those with lower Cu. The negative effect of heavy metal was also observed in other soil enzymes [7,8]. However, Tate II (1987) suggested that at lower concentrations heavy metal may stimulate phosphatase activity in soils[11].

The understanding of the relationship between the activities of phosphatases and heavy metal concentrations in soils is not complete. This is due to the lack of data from some soils, particularly from tropical soils. This research was intended to evaluate the effect of some heavy metals (Pb, Cd, Cu, and Fe) at elevated concentrations of up to 40 mg kg^{-1} on the activities of acid phosphatase in Ultisols from Indonesia (tropical soil) and Japan (temperate soil).

METHODOLOGY

Soil samples were collected from Ap horizons of the Nagoya University Agricultural Farm at Aichi Prefecture, Japan, and of coffee plantation in Sumber Jaja, West Lampung, Indonesia. Both soils were classified as Ultisols. After an air-drying, soil samples were screened to 1 mm and stored at cold room (4°C) prior to use.

A series of 50-ml erlenmeyer flasks each containing 1 g and air-dried soil sample was prefared for the experiment. The soil sample was treated with 50 mg l^{-1} Pb standard-solution diluted with water tc set up a series of Pb addition ranging from 0 to 40 mg kg^{-1} with a soil-to-solution ratio of 1-to-1. The mixtures were swirled for a few seconds to mix the soil and solution and then incubated at room temperature for 1 week. Some series of 50ml erlenmeyer flasks each containing 1 g of air-dried soil sample were also prepared for similiar experiment with Cd, Cu or Fe separated. Analysis of acid phosphatase activities were conducted in the mixtures at the end of the incubation time.

Analysis of acid phosphatase was conducted according to the method of Tabaitabai (1982) with some modification. After completing the incubation time (1 week), 0,2 ml of toluene was added to each flask to stop the microbial activity that might have been producing the soil enzyme; followed by an addition of 4 ml of MUB (modified universal buffer) solution of pH 6.5 and 1 ml of substrate solution i.e. 0.025 M p-nitrophenyl (p-NPP) diluted in MUB of the same pH. After swirling for a few second to mix contains, the mixtures were then incubated in a water bath of 30°C for 11 hours, after which 4 ml of 0.5 M Noah was added to each to stop the enzymatic reaction; followed by an addition of 1 ml of 0.5 M $CaCl_2$ solution to extract p-nitrophenol adsorbed on soil solids. After swirling the flask for a few second to mix the contents, the mixtures were le to stand for several minutes to allow the exchange of the absorbed p-nitrophenol with Ca^{2+}. The p-nitrophenol produced was measured in the filtrate with a Shimadzu UV-2200 UV-Vis Recording Spectrophotometer at λ 400 nm.

Analyses of soil chemical properties including soil pH, total C, total N, and C/N was conducted with conventional methods and soluble heavy-metals (Pb, Cd, Cu, Fe, Mn, Zn) with DTPA method.

RESULT AND DISCUSSION

Soil Heavy Metal and Enzymes

Selected properties of the soil samples are given in Table 1. Except for Fe, the solubilities of heavy metals were generally higher in the Indonesian soil than those in the

Japanese soil. Other than the possible diference in metal sources in the two soils, the differences in the soil pH may reflect this phenomenon. The Indonesian soil showed a pH value almost 1.5 units lower, which may have caused higher solubilities of metal elements in the soil. The activities of all enzymes including acid phosphatese, alkaline phosphatase, urease, and β-glocusidase were higher in the Indonesian soil than in the Japanese soil.

Table 1. Selected Properties of Soil Samples [1)]

Soil Property	Indonesian Soil	Japanese Soil
pH (H_20 1:2)	4.55	5.95
Total C (%)	1.00	1.29
Total N (%)	0.07	0.12
C/N	14.1	10.7
Soluble Metals (mg kg^{-1})		
Pb	3.59	1.11
Cd	0.09	0.07
Cu	2.30	1.86
Fe	70.9	202
Mn	10.8	7.67
Zn	4.44	2.93
Soil Enzymes (μg p-nitrophenol g^{-1} h^{-1}):		
Acid Phosphatase	192	154
Alkaline Phosphatase	25.1	17.5
β-Glucosidase	142	112
Urease[2)]	166	64.2

1). avg of 2. replicates,
2). in μg urea g^{-1} h^{-1}.

Effects of Heavy Metals Elements

Lead (Pb) and Cd, elements north essential to plants, showed a similar effect on the activity of acid phoshatase of both soil (Fig. 1 and 2). The increase in Pb or Cd concentration up to 40 mg kg^{-1} greatly decrease the activity of acid phosphatase. Addition of up to 40 mg kg^{-1} of Cd decreased the acid phosphatase activity as much as 54% In the Japanese soin and 39% in the Indonesian soil. These observation are in agreement with the evidence reported previously [4]. However, this data shows that the activity of acid phosphatase activity was depresses at Pb or Cd addition as low as 15 to 20 mg kg^{-1}. This data also supports the theory suggested by Reddy et al (1987)[7] and Reddy and Faza (1989) [8] that the decrease in enzymatic activities in sewage-sludge treated soils is caused by heavy metal pollutants in the sludge.

Both Pb ad Cd may have decreased the activities of phosphatases trought some possible mechanism. The first possibility was by indirectly depressing the soil microbial activities that produced the soil enzyme during the incubation time and, thereby, decreasing the enzyme activities in soils [2,3]. This possibility is reasonable because of Pb and Cd have been indicated to be toxic to microbial communities [1]. In addition, the magnitude of the element addition was much higher than the element concentration in the soil samples (Table 1).

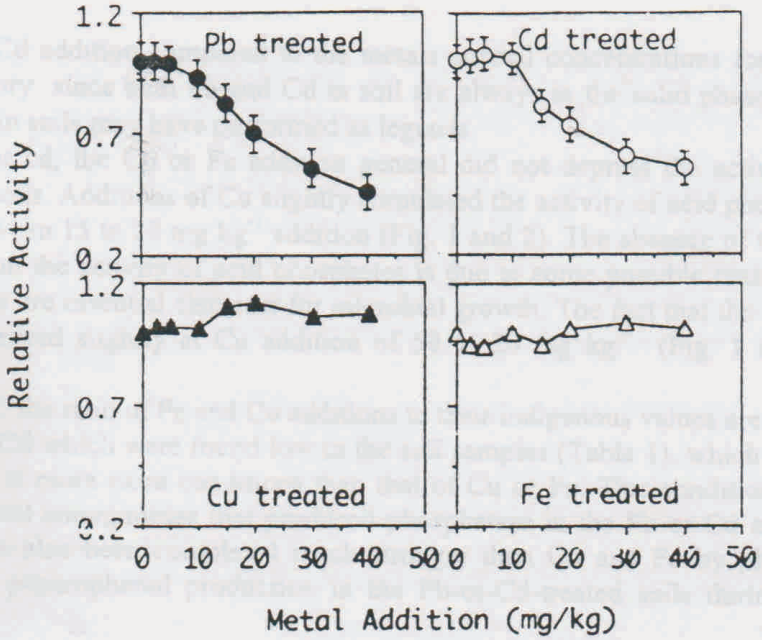

Figure 1. Relative Activity of Acid Phosphatase in the Japanese Soil Treated with Elevated Concentrations of Heavy Metals (Relative Activity is the Activity of Acid Phosphatase Devided by that at Zero Metal Addition).

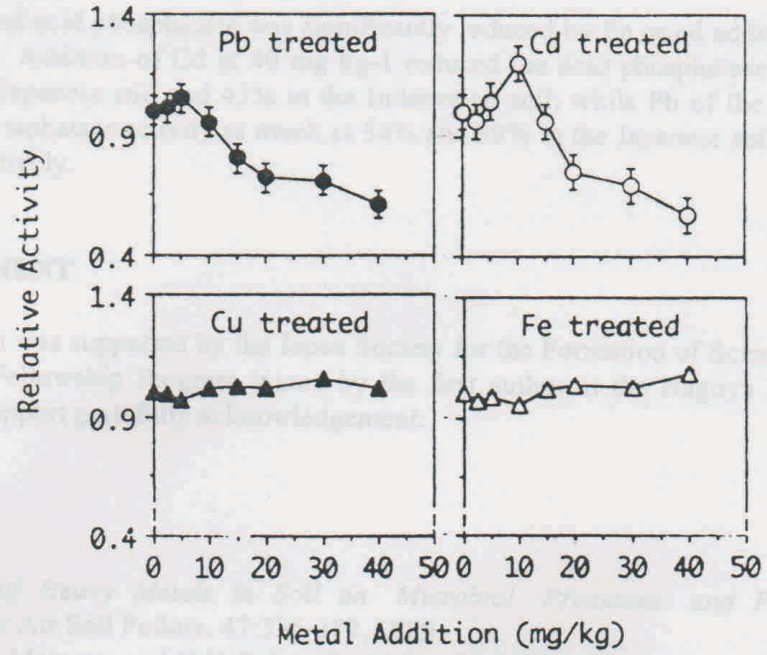

Figure 2. Relative Activity of Acid Phosphatase in the Indonesian Soil Treated with Elevated Concentrations of Heavy Metals (Relative Activity is the Activity of Acid Phosphatase Devided by that at Zero Metal Additions).

The second possibility was that Pb or Cd made concentration complexes with the soil enzymes. The inactivated enzyme complexes could not participate in transformation of P-NPP in producing p-nitrophenol and, thereby, decreased the enzyme activities that could be measured [11]. The mechanism of the coordination of Pb or Cd with acid phosphatase cannot be identified with current data, but it is surely an important possible mechanism. The much higher

magnitude of Pb or Cd addition compared to the metals normal concentrations found in soils may support this theory since both Pb and Cd in soil are always in the solid phases. To some extent, phosphatases in soils may have performed as legends.

Unlike Pb or cd, the Cu or Fe addition general did not depress the activity of acid phosphatase in both soils. Additions of Cu slightly stimulated the activity of acid phosphatase in the Japanese soil between 15 to 20 mg kg^{-1} addition (Fig. 1 and 2). The absence of the negative effect of Cu and Fe on the activity of acid phosphates is due to some possible reasons. Unlike PBS or Cd, Cu and Fe are essential elements for microbial growth. The fact that the activities of acid phosphates increased slightly at Cu addition of 50 to 20 mg kg^{-1} (Fig. 1 and 2) may support this theory.

In additional, the ratio of Fe and Cu additions to their indigenous values are were lower than those of Pb and Cd which were found low in the soil samples (Table 1), which meant that Pb or Cd was added at more extra conditions than that of Cu or Fe. This condition may have depressed the microbial communities that produced phosphatase in the Pb or Cd treated soils. Pb and Cd may have also been completed much stronger than Cu and Fe by phosphatases, resulting in a lower p-nitrophenol production in the Pb-or-Cd-treated soils during the soils essay.

CONCLUSIONS

The activity of acid phosphatase was significantly reduced by Pb or cd addition, but no by Cu or Fe addition. Addition of Cd at 40 mg kg-1 reduced the acid phosphatase activity as much as 44% in the Japanese soil and 43% in the Indonesian soil; while Pb of the same level decreased the acid phosphatase activity as much as 54% and 39% in the Japanese soil and in the Indonesia soil, respectively.

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