Effect of Heavy Metals on Nitrate Reductase Activity of *Eichhornia crassipes* and *Pistia stratiotes*

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Abstract

Effects of eight heavy metals (Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn) on nitrate reductase activity of Echhornia crassipes *and* Pistia stratiotes *were investigated. Plantlets were grown hydroponically in quarter-strength Hoagland's solution and supplemented with 0, 0.1, 0.3, 0.5, 1.0, 3.0 and 5.0 mM of each of the metals for 3 weeks. The results showed that heavy metals, depending on the nature, concentration and duration of exposure inhibit the activity of the enzyme. Low concentration (0.1mM) of zinc activated the enzyme activity in* E. crassipes *while 0.1 and 0.3mM of the same metal boosted the enzyme activity in* P. stratiotes. *Moreover, the inhibitory effect of mercury was most severe.*

Keywords: heavy metals, nitrate reductase activity, *Eichornia crassipes, Pistia stratiotes.*

Introduction

Nitrogen is one of the important mineral elements to plants; hence its metabolism by plants after absorption is an essential phenomenon in plants. Nitrogen is an essential constituent of proteins, nucleic acids and chlorophyll among others. Therefore, processes like protein, nucleic acids and chlorophyll synthesis are related to nitrogen metabolism. Nitrate is considered to be the major source of inorganic nitrogen for most angiosperms (Pilbeam and Kirkby, 1992).

Nitrate absorbed by plants are normally reduced to ammonium before incorporation into nitrogen containing compounds including amino acids (Fan *et al.*, 2002). The first step on the nitrate reduction process is catalyzed by the enzyme nitrate reductase (NR). It is an essential plant enzyme in nitrogen assimilation and was the first recognized example of substrate-inducible enzyme in plants (Lexa *et al.*, 2002). Activity of this enzyme is considered to be a limiting factor for growth, development, and protein production in plants (Solomonson and Barber, 1990). Nitrate reductase activity (NRA) measurements have long been used to indicate the effect of changes in the environment on a plant's capacity to assimilate nitrate nitrogen (Fredeen *et al.*, 1991).

When plants are subjected to stress, their vitality becomes weaker, the longer the stress is maintained. When the limit of the plant's ability to adjust is reached, hitherto latent damage develops into chronic disease or irreversible injury (Larcher, 1981). Heavy metals are among the most dangerous stress factors that has received the attention of environmental biologists in recent times (Larcher, 1995).

Toxic levels of some heavy metals appear as a result of environmental pollution due to the technology of mining, heavy vehicular traffic, smelting, manufacturing, and agricultural

activities in natural and agricultural areas (Oncel *et al.*, 2000). The toxicity of these heavy metals to plants varies with individual metal and concentrations. Induction of leaf chlorosis, reduction of biomass production and nutritional quality have been observed on crops grown in soils contaminated with moderate levels of heavy metals (Clijsters *et al.*, 1999). However, studies particularly of biochemical and molecular levels are required for the subject to be understood.

Since pollution caused by heavy metals is on the increase in the environment, it is necessary to understand how plant metabolisms respond to high levels of heavy metals. Although nitrogen plays a central role in plant metabolism, little information is available regarding the influence of heavy metals on nitrogen metabolism. This paper reported heavy metal-induced changes in nitrate reductase activity of *Eichhornia* crassipes and *Pistia stratiotes*, the two most widespread water plants in Nigerian waters in order to contribute to the existing knowledge regarding this subject in the tropics.

Materials and methods

Plants collection and treatments

Whole plants of *E. crassipes* (Mart) Solms-Laubach and *P. stratiotes* L. were collected from Oba dam, University of Ibadan, Ibadan Oyo State, Nigeria and were washed thoroughly under a running tap water. They were grown and propagated for 4 weeks hydroponically in quarter-strength Hoagland's solution containing (*mM*): 1.25 $Ca(N0_3)_2$, 1.5 $KN0_3$, 0.5 KH_2P0_4, 0.5 $MgS0_4$ and 0.25 NaCl, and (μM): 11.5 H_3BO_3, 2.3 $MnCl_2$, 0.026 H_2MoO_4 and 11.2 Fe-EDTA. Nutrients were replenished at intervals of 5 days throughout the duration of the study. Plants of similar size were selected for the experiment.

Heavy metals were supplied as salts at varying concentrations (0.1, 0.3, 0.5, 1.0, 3.0 and 5.0*mM*) as $AgNO_3$ (Ag), $Cd(NO_3)_2$ (Cd), K_2CrO_4 (Cr), $CuSO_4$ (Cu), $HgCl_2$ (Hg), $NiSO_4$ (Ni), $Pb(NO_3)_2$ (Pb) and $ZnSO_4$ (Zn). Nutrient solution devoid of any of these metals served as control. Both the control and the treated solutions were maintained at pH 5.5 using 0.5*M* of either HCl or NaOH.

Experimental plants (in triplicates) were placed in nutrient solution (1 litre) supplemented with one of the metals under investigation. The experimental set-up was maintained for 3 weeks in a screen house, nursery section of the Department of Botany and Microbiology, University of Ibadan. The substrate assay solution contained 1 ml each of 0.1M KNO_3, 15 ml l^{-1} propan-1-ol and 0.1M potassium phosphate buffer (pH 7.5)

Collection and Preparation of Homogenates

Fresh leaves of plants were harvested weekly and thoroughly washed under a running tap water. Homogenates of leaves were prepared by grinding fresh leaves (0.5g) in distilled water (10 ml) followed by filtration using Labsman No 1 filter paper. The filtrate was utilized for enzyme assays (De Biasi *et al.*, 2003).

Assay of Nitrate Reductase Activity

Assay of nitrate reductase activity was performed following the method described by Fan *et al.* (2002). Homogenates (1 ml) was mixed with the substrate (3ml) followed by incubation at room temperature (26ºC) in the dark for 1hour in a test tube. Thereafter, 1ml of the reaction mixture was transferred into a clean test tube, and 1ml 1% (w/v) sulphanilic acid (1 ml) and 0.2M naphthylenediamine (1 ml) were added. The reaction mixture was thoroughly shaken and left to stand for 1 hour for colour formation. The blank contained substrate (1 ml), 1% (w/v) sulphanilic acid (1ml) and 1ml of 0.2M naphthylenediamine. The reaction mixture was allowed to stand for 1 hour for colour formation. The absorbance was read at 540 nm on Cam-Spec M105 spectrophotometer. The values were obtained from a standard calibration curve generated using solutions of Sodium nitrite. One unit of nitrate reductase activity was defined as µmol NO_2 produced per gram fresh weight per hour. Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as a standard.

Statistical analysis

Data analyses were performed using SAS version 6.0 (SAS Institute, 1989). Analysis of variance (ANOVA) was performed on experimental data. For mean separations, Duncan's Multiple Range Test (DMRT) was used at $P \leq 0.05$ (Little and Hill, 1978).

Results and Discussion

Nitrate reductase activity was significantly ($P \leq 0.05$) inhibited at metals concentrations ≥0.3*mM*. This observation was true for all the metals except zinc. Low level of Zn (0.1*mM*) induced higher level of NRA when compared with the control *E. crassipes* while 0.1 and 0.3*mM* Zn concentrations induced higher level of the enzyme activity than the control *P. stratiotes*. It was observed that, enzyme activity decreased with increase in metal concentrations and the duration of exposure. *E. crassipes* and *P. stratiotes* treated with 0.5*mM* Ag had their NRA reduced from 5.13±0.13 and 3.90±0.31 µmol g^{-1} fresh leaf weight at the end of the first week to 4.07±0.52 and 2.90±0.32 µmol g^{-1} fresh leaf weights respectively at the end of the third week. The control *E. crassipes* however exhibited reduced enzyme activity during the second week and increased significantly during the third week (Figs. 1 and 2). The control nitrate reductase activity in *E. crassipes* was 13.47±0.35 µmol g^{-1} fresh leaf weight. This value is significantly ($P \leq 0.05$) greater than 7.27±0.55, 9.13±0.29, 8.87±0.24 and 0.83±0.12 µmol g^{-1} fresh leaf weight recorded for plants treated with 0.3*mM* Ag, Cd, Cu and Hg (Figs.1 and 2). *Pistia stratiotes* exposed to 0.3*mM* Ag, Cr, Hg, Ni and Pb had NRA values of 5.80±0.21, 9.33±0.19, 2.23±0.09, 3.17±0.12 and 7.10±0.10 µmol g^{-1} fresh weight respectively. These values were significantly ($P \leq 0.05$) lower than 10.17±0.26 µmol g^{-1} fresh weight obtained for the control (Figs. 3 and 4).

The increase in nitrate reductase activity compared to the control plants caused by the low concentrations of Zn could be attributed to the ability of Zn to provide a more balanced nutrient requirement for optimum vitality of the plants. The decrease in nitrate reductase activity at the second week of the experiment could be as a result of stress caused by inadequate nutrients in the medium. The interaction of these heavy metals with the enzyme could be as a result of the metals binding to the active sites of the enzyme involved in the catalytic activity or structural integrity of the enzyme. The results of the present study have shown that the effect heavy metal has on nitrogen metabolism is dependent on the nature of the metal, the concentration to which the plant was exposed and the duration of exposure. Conclusively, high levels of these metals inhibit nitrate reductase activity in *E. crassipes* and *P. stratiotes*, therefore their abilities to metabolize absorbed nitrogen were greatly hindered.

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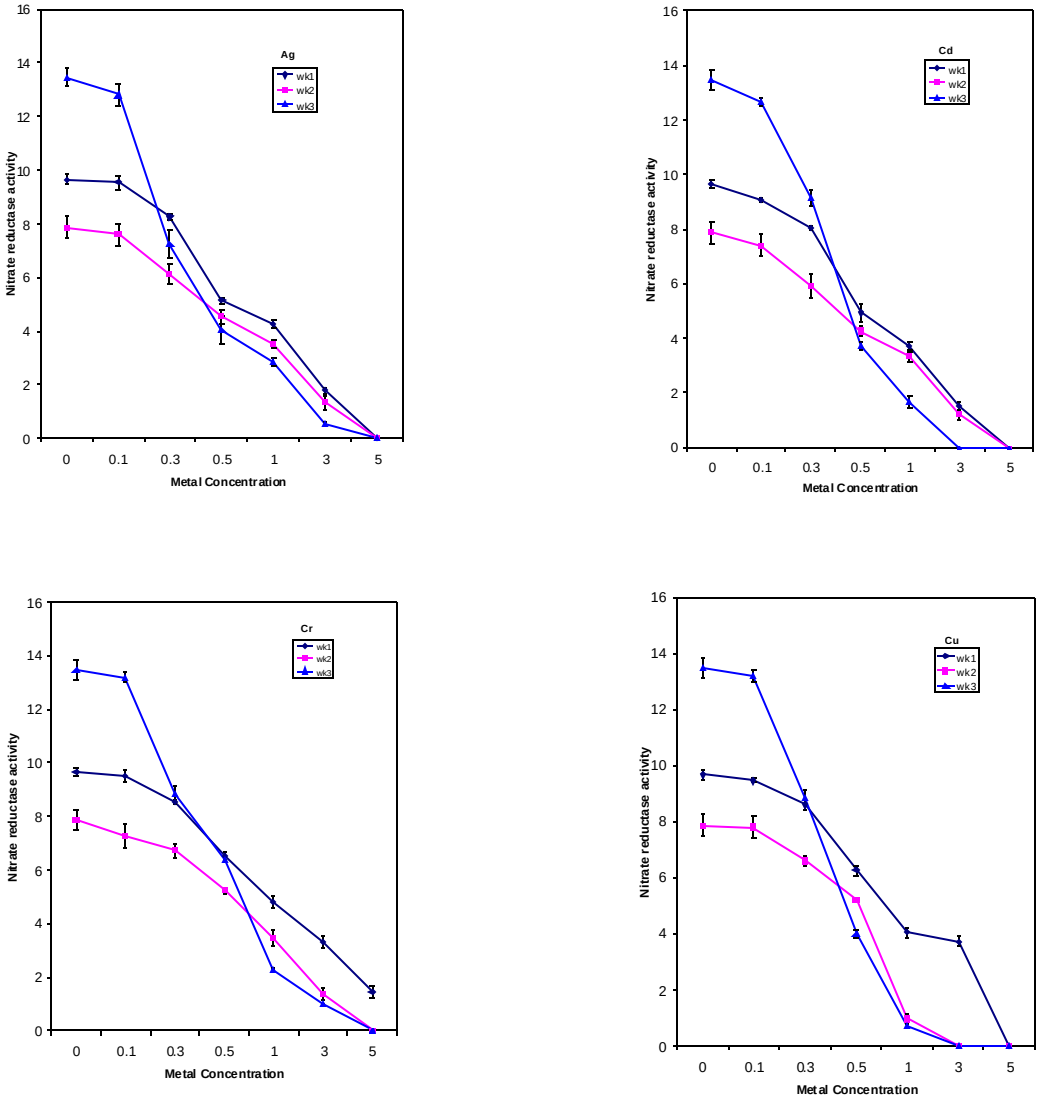

Figure 1: Nitrate reductase activity (μmol g^{-1} fresh weight) of *E. crassipes* treated with Ag, Cd, Cr and Cu.

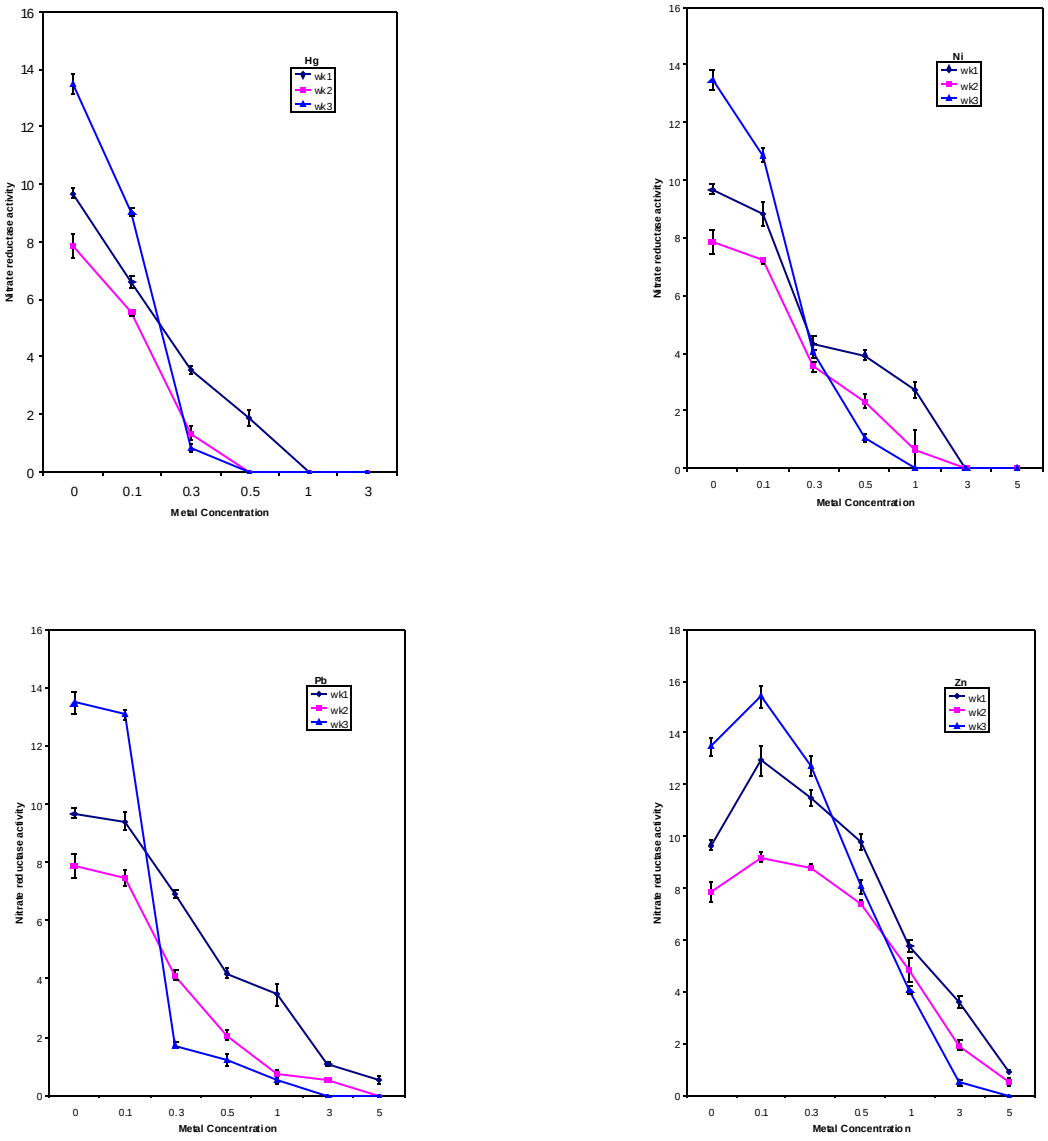

Figure 2: Nitrate reductase activity (μmol g^{-1} fresh weight) of *E. crassipes* treated with Hg, Ni, Pb and Zn.

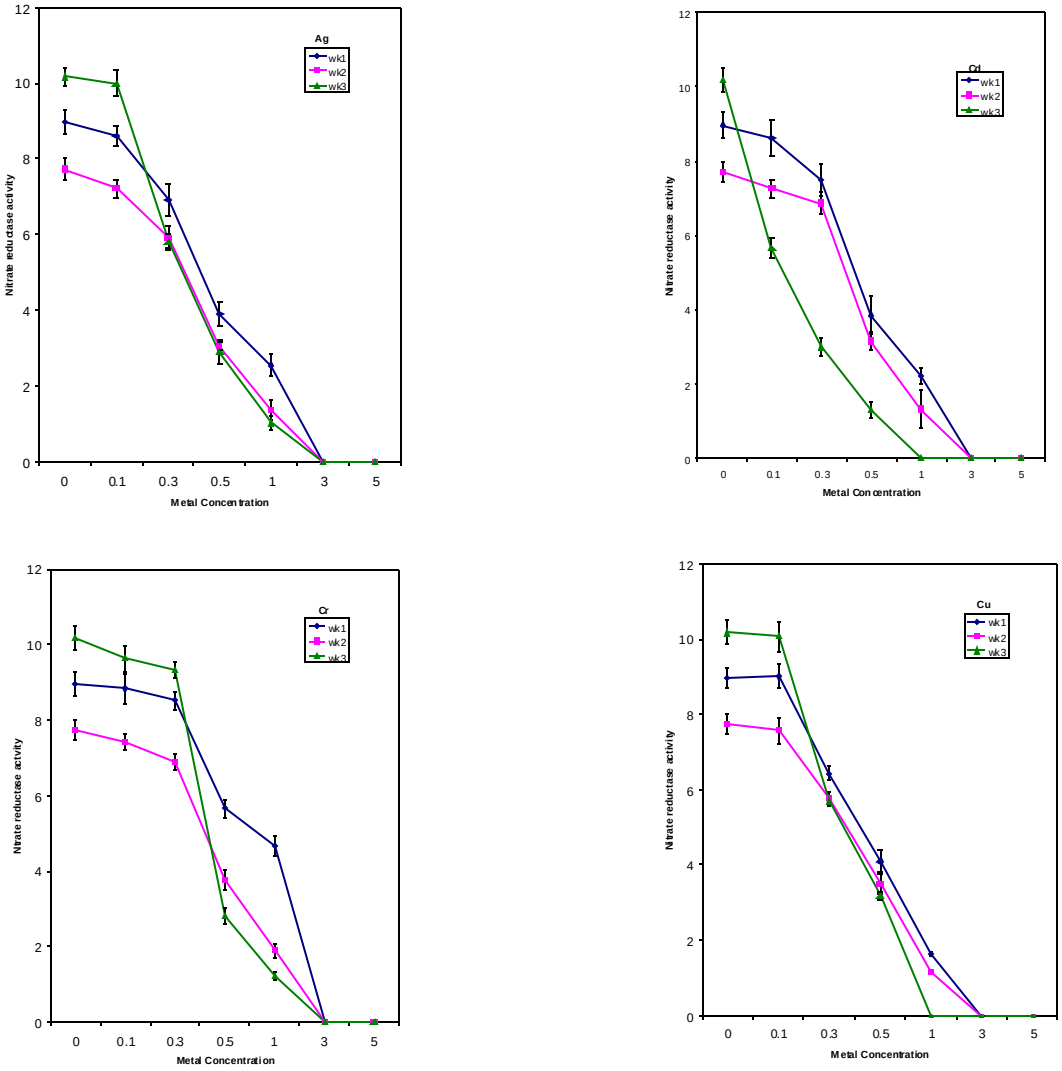

Figure 3: Nitrate reductase activity (µmol g^{-1} fresh weight) of *P. stratiotes* treated with Ag, Cd, Cr and Cu.

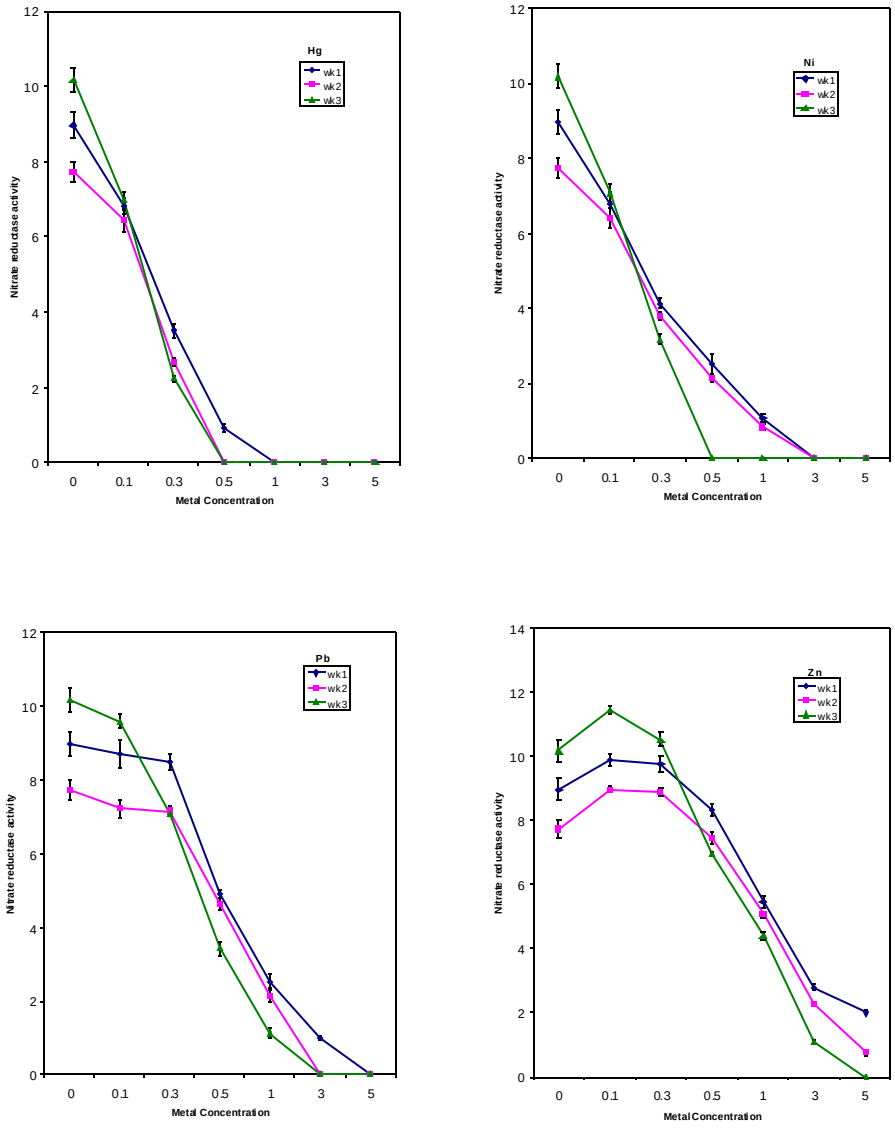

Figure 4: Nitrate reductase activity (µmol g^{-1} fresh weight) of *P. stratiotes* treated with Hg, Ni, Pb and Zn.