Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

| ISSN: 0972-6268 | Vol. 10 | No. 4 | pp. 505-510 | 2011 |
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Original Research Paper

# Amelioration of Toxic Effects of Lead in *Vigna radiata* (L.) Wilczek with the Application of *Pseudomonas fluorescens*

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**Nat. Env. & Poll. Tech.**
**Website: www.neptjournal.com**

*Received:* 17/3/2011
*Accepted:* 30/4/2011

**Key Words:**
Heavy metals, Lead
*Vigna radiata*
Bioremediation
*Pseudomonas fluorescens*

## ABSTRACT

Pollution by heavy metals is one of the important threatening factors in the world. The present study was intended to analyse the effect of lead (Pb) on seed germination, growth and chlorophyll content of *Vigna radiata* (L). Wilczek. Germination parameters like germination percentage, radicle and hypocotyl length, vigour index and growth index were studied, and found to be decreased with increasing concentrations. Phytotoxicity level increased at elevated concentrations. At higher concentrations, severe morphological symptoms and reduction in chlorophyll content were observed. However, by treatment with *Pseudomonas fluorescens,* a plant growth promoting rhizobacterium, a significant increase in all parameters was observed.

## INTRODUCTION

Rapid industrialisation and urbanisation in the last century have led to the problems of environmental pollution and ecological damage. Indiscriminate and excessive use of synthetic fertilizers and chemicals have damaged the ecosystems considerably.

The ecological consequences of prolonged overuse of chemicals including heavy metals, on soil include decline in soil fertility, soil salination due to salt accumulation, water logging, deterioration of the environment, human health hazards, and poor sustainability of agriculture lands.

Heavy metals are conventionally defined as elements with metallic properties with atomic number of more than 20. A number of heavy metals are required by plants as micronutrients. Excessive accumulation of heavy metals is toxic to most plants. Heavy metal ions, when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot, leading to impaired metabolism and reduced growth. Heavy metal contamination of water and soil poses major environmental and human health problems.

Among the heavy metals, lead particularly has become a cosmopolitan environmental pollutant (Sharma & Dubey 2005). It is prevalent in the natural environment. Main sources of lead pollution include mining and smelting activities, use of lead containing paints and drainage of untreated municipal sewage (Chaney & Ryan 1994). It is a non essential element for plants but shows high tendency for its uptake and accumulation in different plant organs (Sharma & Dubey 1994). Toxic effects of lead on plants grown on lead contaminated soils include inhibition of photosynthesis, deficient mineral nutrition uptake and the problem of water imbalance, which considerably reduce both the vegetative and reproductive growth of plants (Johnson & Eaton 1980).

To reduce pollution of ecosystems and cleanup the environment, scientists are in search of breakthrough, and phytoremediation is a relatively new approach for removing contaminants from environment. Phytoremediation may be defined as the use of plants to remove, destroy or sequester hazardous substances from the environment. Unfortunately, even plants are relatively tolerant to various environmental contaminants, but their growth become stunted. To remedy this situation, plant growth-promoting bacteria, which facilitate growth of plants, especially under environmental stressful conditions, may be added to roots of the plants. These bacteria lower the level of growth inhibiting stress ethylene within the plants and also provide the plant with iron from the soil. The net result of adding these bacteria to plants is a significant increase in both number of seeds that germinate and the amount of biomass that the plants are able to attain. This makes phytoremediation a much faster and more efficient process in presence of plant growth promoting bacteria. Typically bioremediation provides an efficient and economical way to reduce environmental toxins using indigenous or introduced microbes that naturally degrade contaminants. The major advantage of bioremediation is that it is a natural process and can be used at much lower cost than many other treatment technologies.

*Pseudomonas fluorescens* encompasses a group of common, non-pathogenic saprophytic bacteria, which colonize soil, water and plant surface environments. It secretes plant growth promoting hormones like cytokinin and IAA leading to plant growth stimulation. *Pseudomonas* are noted for their metabolic diversity and often isolated from environments designed to detoxify bacteria that degrade pollutants. Bioremediation application seek to exploit the inherent metabolic diversity of *Pseudomonas fluorescens.*

In this industrialised world the pollution study on plants has its own relevance. Present study deals with the impact of lead on the growth of *Vigna radiata* (L). Wilczek and its remediation using *Pseudomonas fluorescens.*

## MATERIALS AND METHODS

The plant material selected for the present study is *Vigna radiata* (L) .Wilczek of the family Leguminosae. The seeds were obtained from Kerala Agriculture University, Mannuthy. The viable seeds selected were used for germination studies and the seedlings were used for morphological and biochemical analysis.

Lead was applied in the form of lead nitrate and the solutions were prepared in distilled water. The selected concentrations of the metal were in the range of 10mM to 50mM. *Pseudomonas fluorescens* for bioremediation was also collected from Kerala Agriculture University, Mannuthy. The parameters used for the study were:

**1. Germination studies:** a. Germination percentage, b. Vigour index, c. Phytotoxicity, d. Growth index

**2. Morphological studies:** a. Height of the plant, b. Length of the root, c. Number and size of leaves, d. Leaf area, e. Net productivity, f. Stomatal frequency

**3. Biochemical studies:** Quantitative estimation of chlorophyll.

All the parameters were studied and compared with control.

**Germination study:** The germination study was conducted in April. The seeds were maintained under room temperature for 24 hours. Sterilized Petri dishes were used for this treatment and the concentration of lead taken were 10mM, 20mM, 30mM, 40mM and 50mM.

Surface sterilized seeds were soaked for 12 hours in the respective concentrations of lead, and kept in sterilized Petri dishes, lined with Whatman No.1 filter paper, for germination. Filter papers were moistened with the solutions. Thin layer of cotton was used for keeping moisture. The control seeds were raised in distilled water. Triplicates were maintained for each treatment. The treated seeds were kept under observations for 5 days. Emergence of radicle was considered as germination. The percentage of germination and radicle-hypocotyl length were noted from first day to fifth day of the experiment.

Again, the fresh seeds were soaked in *Pseudomonas fluorescens,* a biofertilizer, for 12 hours. These seeds were also treated with different concentrations of lead solution. The germination parameters were studied and compared with the control.

**Morphological study:** The surface sterilized seeds were equispatially arranged in polythene bags containing 3 kg of soil. The bags were irrigated with equal volumes of different concentrations of lead solution. Control sets were irrigated with equal volume of distilled water. The morphological parameters and biochemical contents were analysed on 15th day.

The above experiments were also carried out with the seeds and seedlings treated with *Pseudomonas fluorescens*. Morphological parameters like height of plants, length of root, number and size of leaves, leaf area index, net productivity were studied in different concentrations and compared with control. Fresh weight (g/plant) was taken with the help of an electrical balance. The collected plant materials were kept in hot air oven at 80°C for 24 hours and their dry weights (g/plant) were taken.

## RESULTS AND DISCUSSION

The results of the study are presented in Tables 1-6 and Figs. 1-11. The present study was conducted to determine the effect of lead on the growth of *Vigna radiata* and its bioremediation by *Pseudomonas fluorescens*. The results showed a significant reflection of the plant towards the heavy metal throughout the germination, and morphological and chlorophyll contents. Data presented in Table 1 show that higher concentration of lead results in a progressive decrease of percentage germination as compared to the control. At lower concentrations lead does not produce any severe symptoms. This result was in agreement with the experiments of Maiti et al. (2002). All growth parameters were reduced with increasing concentrations of lead. A severe effect is clearly seen in radicle of seedling than hypocotyl. The complete inhibition of lateral root and root hair was observed in higher concentrations. Inhibition of root growth, lateral root and root hair formation can be explained by the parallel works of Tomar et al. (2000) and Piechalk et al. (2002).

In morphological studies, the metal treatment showed a reduction in all growth parameters in *Vigna radiata.* The higher impact of lead was observed in the root growth as compared to shoot leading to the higher reduction in its length and fresh weight. Many workers found that lead toxicity was affected more in roots of plants than shoots (Ewais 1997,

Table 1: Effect of Pb on germination studies in *Vigna radiata.*

| Sl.No. | Concentration (mM) | Germination (%) | Vigour index | Radicle length (cm) | Hypocotyl length (cm) | Growth index (cm) | Phytotoxicity (%) |
|---|---|---|---|---|---|---|---|
| 1 | Control | 100.00% | 484 | 4.08 | 4.84 | 8.92 | Nil |
| 2 | 10mM | 85.33% | 148.6 | 1.45 | 1.72 | 0.34 | 67.40% |
| 3 | 20mM | 77.33% | 76.84 | 1.04 | 1.12 | 0.21 | 77.45% |
| 4 | 30mM | 66.66% | 86.2 | 0.94 | 1.23 | 0.2 | 76.22% |
| 5 | 40mM | 42.66% | 29.86 | 0.83 | 0.7 | 0.09 | 79.65% |
| 6 | 50mM | 14.66% | 7.33 | 0.6 | 0.5 | 0.07 | 84.31% |

Table 2: Effect of Pb on the morphological parameters in *Vigna radiata.*

| Sl.No. | Concentrations(mM) | Plant height (cm) | Length of root (cm) | No. of leaves | Size of leaves (cm) | Net productivity (g/day) | Stomatal index |
|---|---|---|---|---|---|---|---|
| 1 | Control | 21 | 4.5 | 3 | 3.19 | 0.0360 | 0.446 |
| 2 | 10mM | 20 | 3.5 | 2 | 3.2 | 0.0240 | 0.457 |
| 3 | 20mM | 19 | 2.5 | 2 | 2.5 | 0.0220 | 0.480 |
| 4 | 30mM | 15 | 2.1 | 2 | 2.26 | 0.0200 | 0.500 |
| 5 | 40mM | 9 | 1.5 | 2 | 1.91 | 0.0160 | 0.520 |
| 6 | 50mM | 7 | 1.1 | 2 | 1.7 | 0.0140 | 0.530 |

Table 3: Effect of Pb on the chlorophyll content in V*igna radiata.*

| Sl. No. | Concentrations (mM) | Chlorophyll '*a*'(mg/g fr.wt) | Chlorophyll '*b*'(mg/g fr.wt) | Total chlorophyll (mg/g fr.wt) |
|---|---|---|---|---|
| 1 | Control | 0.3172 | 0.3350 | 0.6500 |
| 2 | 10mM | 0.3170 | 0.3200 | 0.6400 |
| 3 | 20mM | 0.2780 | 0.3000 | 0.5800 |
| 4 | 30mM | 0.2032 | 0.2100 | 0.4100 |
| 5 | 40mM | 0.1829 | 0.1900 | 0.3700 |
| 6 | 50mM | 0.1720 | 0.1900 | 0.3600 |

Piechalk et al. 2001). According to Sharma & Dubey (1994) and Tomar et al. (2000), lead can cause multiple direct and indirect effects on plant growth metabolism, along with visible symptoms including stunted growth and small leaves leading to membrane disorganisation and reduced photosynthesis. In this study, some plants show improved shoot length in all the concentrations. It may be due to the enhanced availability of nitrogen in shoots as lead nitrate. It was reported in *Lycopersicon esculentis* that increasing levels of lead invariably decrease the root nitrogen significantly, while shoot nitrogen increased generally with lower doses of metal addition. The number of stomata were found to be increased with increasing concentrations, but stomata showed smaller size than control. The same effect was reported by Tomar et al. (2000) in *Vigna radiata* and Kastori et al. (1992) in *Helianthus annus*.

Chlorophyll content is one of the important biochemical parameters, which is used as the index of production capacity. In this study Chl. *a*, Chl. *b* and total chlorophyll were reduced under lead treatment at higher concentrations. The similar findings were reported by many other workers (Plesnicar 1998, Maiti et al. 2002, Rakesh Singh et al. 2008). The decrease in photosynthetic pigment was due to lead, which resulted in decreased supply of photosynthetic products to the actively growing organs suppressing growth (Fargasoa 1998). The decline in chlorophyll content in plants exposed to lead stress is believed to be due to inhibition of enzymes (Padmaja et al. 1990) and protochlorophyllide reductase associated with chlorophyll biosynthesis impairment in the supply of $Mg^{2+}$, $Fe^{2+}$ and $Zn^{2+}$ (Kupper et al. 1996 ).

Table 4: Effect of Pb on germination parameters of *Vigna radiata* after treatment with *Pseudomonas fluorescens.*

| Sl.No. | Concentration (mM) | Germination (%) | Vigour index | Radicle length (cm) | Hypocotyl length (cm) | Growth index (cm) | Phytotoxicity (%) |
|---|---|---|---|---|---|---|---|
| 1 | Control | 100 | 500 | 4.6 | 5 | 1 | - |
| 2 | 10mM | 100 | 200 | 1.83 | 2 | 0.39 | 0.60% |
| 3 | 20mM | 100 | 161 | 1.51 | 1.61 | 0.32 | 0.67% |
| 4 | 30mM | 100 | 137 | 1.26 | 1.37 | 0.27 | 0.72% |
| 5 | 40mM | 78.66 | 62.92 | 1.05 | 0.8 | 0.19 | 0.77% |
| 6 | 50mM | 76 | 48 | 0.77 | 0.6 | 0.14 | 0.83% |

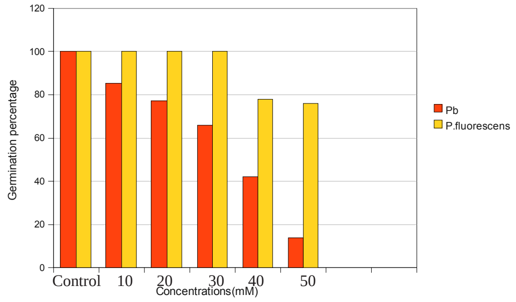


Fig. 1: Effect of Pb on germination percentage of *V. radiata* before and after traeted with *P. fluorescens*.

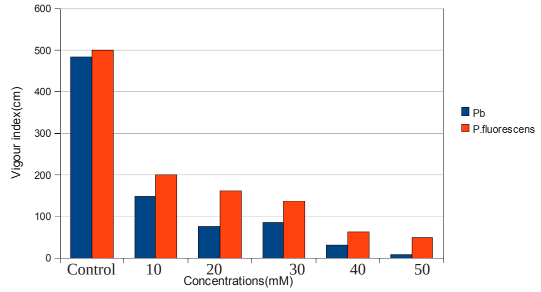


Fig. 2: Effect of Pb on vigour index of *V. radiata* before and after traeted with *P. fluorescens*.

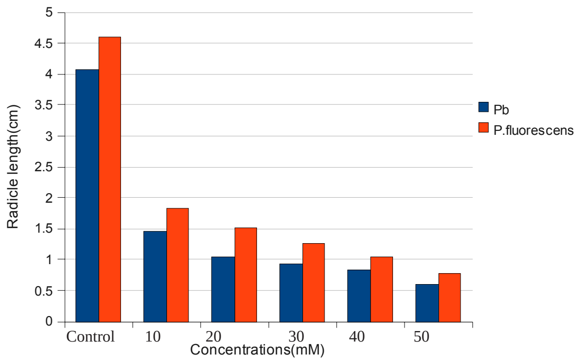


Fig. 3: Effect of Pb on radicle length of *V. radiata* before and after traeted with *P. fluorescens*.

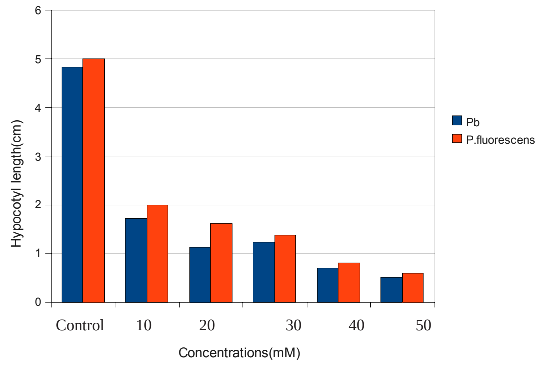


Fig. 4: Effect of Pb on hypocotyl length of *V. radiata* before and after traeted with *P. fluorescens*.

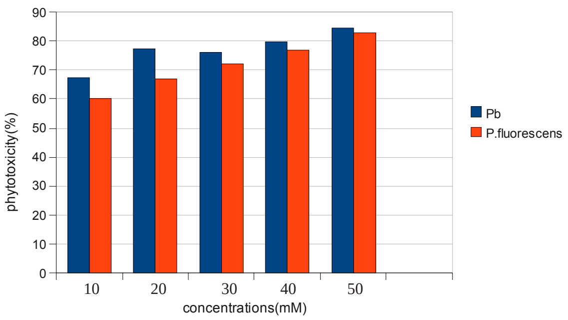


Fig. 5: Effect of Pb on phytotoxicity of *V. radiata* before and after traeted with *P. fluorescens*.

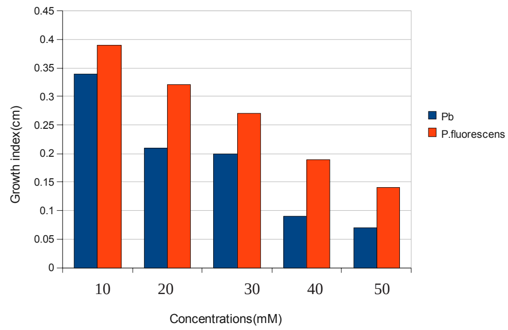


Fig. 6: Effect of Pb on growth index of *V. radiata* before and after traeted with *P. fluorescens*.

Table 5: Effect of Pb on morphological parameters of V*igna radiata* after treatment with *Pseudomonas fluorescens.*

| Sl.No. | Concentra-tions(mM) | Plant height (cm) | Length of root (cm) | No. of leaves | Size of leaves (cm) | Net produc-tivity (g/day) | Stomatal index |
|---|---|---|---|---|---|---|---|
| 1 | Control | 24 | 5 | 3 | 3.19 | .038 | 0.445 |
| 2 | 10mM | 23 | 4 | 3 | 3.2 | .025 | 0.447 |
| 3 | 20mM | 20 | 3.1 | 2 | 2.5 | .024 | 0.473 |
| 4 | 30mM | 15 | 2.3 | 2 | 2.26 | .023 | 0.493 |
| 5 | 40mM | 9 | 1.8 | 2 | 1.91 | .022 | 0.508 |
| 6 | 50mM | 7 | 1.5 | 2 | 1.7 | .021 | 0.521 |

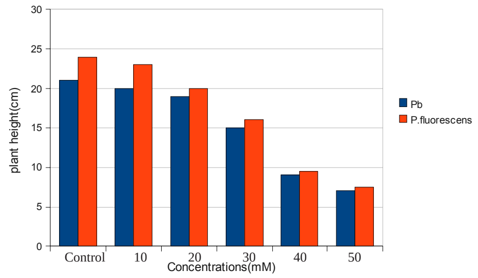


Fig. 7: Effect of Pb on plant height of *V. radiata* before and after traeted with *P. fluorescens*.

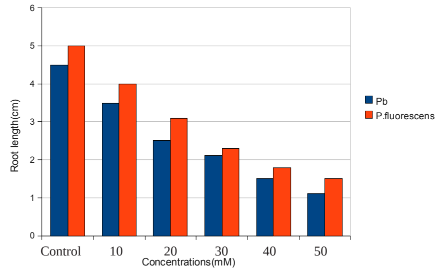


Fig. 8: Effect of Pb on root length of *V. radiata* before and after traeted with *P. fluorescens*.

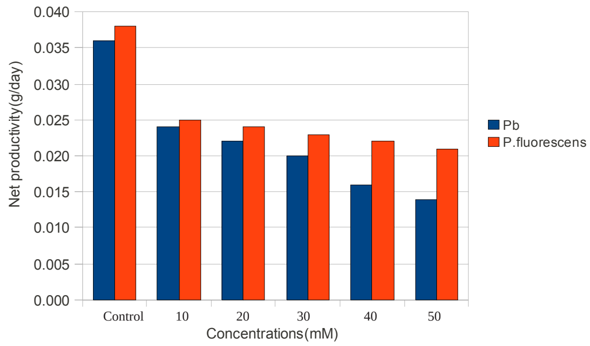


Fig. 9: Effect of Pb on net productivity of *V. radiata* before and after traeted with *P. fluorescens*.

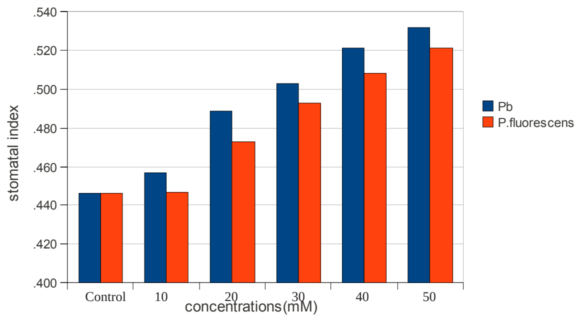


Fig. 10: Effect of Pb on stomatal index of *V. radiata* before and after traeted with *P. fluorescens*.

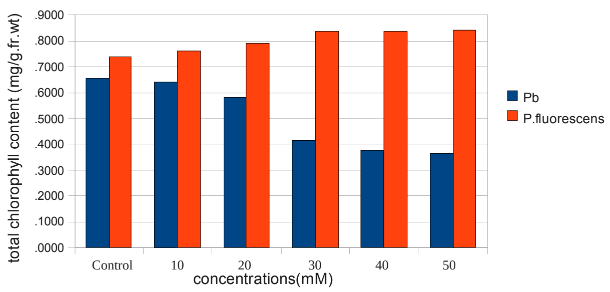


Fig. 11: Effect of Pb on total chlorophyll content of *V. radiata* before and after traeted with *P. fluorescens*.

Table 6: Effect of Pb on the chlorophyll content in *Vigna radiata* after treatment with *Pseudomonas fluorescens.*

| Sl. No. | Concentr-ations (mM) | Chlorophyll '*a*'(mg/g fr.wt) | Chlorophyll '*b*'(mg/g fr.wt) | Total chlorophyll (mg/g fr.wt) |
|---|---|---|---|---|
| 1 | Control | 0.3386 | 0.3579 | 0.6965 |
| 2 | 10mM | 0.3190 | 0.3470 | 0.6660 |
| 3 | 20mM | 0.3204 | 0.3450 | 0.6654 |
| 4 | 30mM | 0.3102 | 0.3417 | 0.6519 |
| 5 | 40mM | 0.3200 | 0.3308 | 0.6508 |
| 6 | 50mM | 0.3114 | 0.3314 | 0.6428 |

On the other hand, inhibitory effects of lead on growth parameters were decreased by the treatment with *Pseudomonas fluorescens.* It showed an improvement in seed germination, radicle length, vigour index, etc. Singh et al. (2010) reported that in *Vigna radiata* rhizobacterial strains helped in ameliorating metal induced phytotoxicity acquiring higher biomass and metal uptake in the plant. The plant may be useful in decontamination of metals from polluted soil. Also some workers reported that joint activity of plant and plant hormones like gibberellic acid and kinetin reduce the metal toxicity caused by heavy metals (Abdel Haleem et al. 2007, (Mumtaz Hussain et al. 2006). It is also found that *Pseudomonas fluorescens* produces plant hormones like cytokinin and IAA, which help the growth of plants. *Pseudomonas fluorescens* is also a plant growth promoting rhizobacterium.

Over all, highly toxic effects of lead on germination, growth and photosynthetic pigment were observed in this study. Moreover, *Pseudomonas fluorescens* proved that it reduces the effects of lead and increases the growth of plants.

From the above discussion, it can be concluded that the lead at high concentration severely affect the germination, growth and chlorophyll content in *Vigna radiata.* At lower concentration, the plants showed tolerance. Also the treatment with *Pseudomonas fluorescens* showed positive results in all the growth parameters. It reveals that *Pseudomonas* *fluorescens* can be used to improve the growth of plants under heavy metal stress. It is also recommended that *Pseudomonas fluorescens* can be used for bioremediation process.

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