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Heavy Metal Inhibition of in vitro *Rhizobium* Growth, Nodulation and Nitrogen Fixation in *Vigna radiata*

By

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With 2 Figures

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Summary

Bhandal I. S., Kaur H. & Bhandal B. 1990. Heavy metal inhibition of in vitro *Rhizobium* growth, nodulation and nitrogen fixation in *Vigna radiata.* – Phyton (Horn, Austria) 30 (1): 57–63, 4 figures. – English with German summary.

Four different heavy metal salts e. g. $NiCl_2$, $Pb(NO_3)_2$, $CdSO_4$ and $CuSO_4$ were tested for their effect on in vitro *Rhizobium* growth, nodulation and acetylene reduction activity of the nodules in *Vigna radiata* Wilczek cv. ML 20 *(Fabaceae).* Treatment with $CdSO_4$ and $NiCl_2$ had the most deleterious effect on the growth of *Rhizobium,* whereas, no significant effect was observed in case of $Pb(NO_3)_2$ and $CuSO_4$. Nodulation parameters e. g. number of plants nodulated, number of nodules per plant, number of nodules per g fresh weight of the roots, nodule dry weight per plant and weight of the nodule were inhibited, the inhibition being concentration dependent. The effect was identical in all the heavy metal salts tested. The heavy metal salts except $NiCl_2$ also caused a decrease in the acetylene reduction activity of the nodules. Inhibition of nodulation has been suggested to be due to the inhibitory effect of the heavy metal salts on the growth of *Rhizobium* as well as on the less number of infection sites available.

Zusammenfassung

Bhandal I. S., Kaur H. & Bhandal B. 1990. Schwermetall-Hemmung des in vitro-Wachstums von *Rhizobium*, der Knöllchenbildung und der Stickstoffbindung bei *Vigna radiata.* – Phyton (Horn, Austria) 30 (1): 57–63, 4 Abbildungen – Englisch mit deutscher Zusammenfassung.

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Die Schwermetalle Nickel (als Chlorid), Blei (als Nitrat) sowie Cadmium und Kupfer (als Sulfat) wurden auf ihre Wirkung auf das in vitro-Wachstum von *Rhizobium,* auf die Knöllchenbildung und die Azetylenreduktion an *Vigna radiata* WILCZEK cv. ML 20 *(Fabaceae)* getestet. $CdSO_4$ und $NiCl_2$ hemmten das Wachstum von *Rhizobium* am stärksten, mit Pb $(NO_3)_2$ und $CuSO_4$ wurde keine signifikante Wirkung beobachtet. Die Knöllchenbildung an den Wurzeln und das Knöllchengewicht wurde durch alle vier untersuchten Schwermetalle konzentrationsabhängig gehemmt. Mit Ausnahme von Nickel hemmten die Metalle die Azetylenreduktion durch die Knöllchen. Die verminderte Nodulation wird teils auf das durch die Schwermetalle gehemmte Wachstum von *Rhizobium,* teils auf die verminderte Zahl geeigneter Infektionsorte zurückgeführt.

Introduction

Industrial effluents are causing considerable pollution of our land and water resources. Accumulation of heavy metal ions in the soil has been reported to be toxic to the free-living microorganisms (BABICH & STOTZKY 1985). Some of these free-living microbes (nitrogen fixing bacteria and blue green algae) convert gaseous nitrogen into organic nitrogen which is used by the plants as nitrogen source. Other nitrogen fixing bacteria have a symbiotic relationship with the leguminous plants. The establishment of symbiosis i. e. root nodulation is an orderly process and is also influenced by the edaphic factors. Only a few reports are available dealing with the toxicity of different heavy metals in nodulation and nitrogen fixation (HUANG & al. 1974, MCILVEEN & COLE 1974, DECARVALHO & al. 1982, PAIVOKE 1983 a b, YAKOLEVA 1984). Present studies were undertaken to investigate the role of Cd^{27}, Ni^{2+}, Pb^{2+} and Cu^{2+} on the growth of *Rhizobium* as well as on nodulation and acetylene reduction in Modules of *Vigna radiata.*

Materials and Methods

Different concentrations of nickel chloride ($NiCl_2 \cdot 6H_2O$), lead nitrate ($Pb(NO_3)_2$, cadmium sulphate ($CdSO_4 \cdot 7H_2O$) and copper sulphate ($CuSO_4 \cdot 5H_2O$) were added into 50 ml of the YEMA bacterial growth medium afte autoclaving. The cultures were inoculated with one ml of an overnight bacterial culture (*Rhizobium* sp. Cowpea group, strain M_1). The cultures were incubated at 25° C on a shaker. After four days of incubation 3 ml aliquot of the culture medium was withdrawn and its optical density was recorded at 580 nm.

Vigna radiata WILCZEK cv. ML 20 were grown in earthen pots containing 3 kg of garden soil. The soil was treated with heavy metal salts by adding 150 ml solution of heavy metal salts per 3 kg of soil. The heavy metal salt concentration was calculated on soil weight basis. The soil was thoroughly mixed and allowed to equilibrate for a week. The treated soil was then put in the pots. Each treatment carried three replicates. The seeds were surface sterilized with 0.1% $HgCl_2$ solution for 10 min. followed by rinsing with distilled water. The seeds were then incubated for 1 h with an overnight culture of *Rhizobium* sp. strain M_1 at 30° C. Treated weeds were sown in the pots. The pots were irrigated with water on alternate days and with nutrient solution (JENSEN 1942) after every fifteen days. Only three seedlings per pot were retained after the establishment of the seedlings. The plants were maintained in the green house.

After 45 days of growth the plants were removed from the pots along with the soil. The soil was carefully removed so as to prevent damage to the root growth. The root system was cut at the base of the stem and was processed immediately for the assay of acetylene reduction activity by the method of HARDY & al. 1968. After assay of the enzyme nodulation parameters viz. number of plants nodulated per treatment, number of nodules per plant, number of nodules per g fresh weight of root and nodule dry weight per plant were recorded. Dry weight per nodule was calculated from this data.

Results and Discussion

Addition of heavy metal salts to the bacterial culture medium drastically reduced the growth of the *Rhizobium* except in case of $Pb(NO_3)_2$ and $CuSO_4$ treatment (Table 1). Cadmium sulphate was the most toxic of the heavy metal salts tested. Absence of any effect of Pb may be due to the precipitation of the metal salt in the growth medium and thus its conversion into unavailable form. In case of $CuSO_4$, the results are in agreement with the known essentiality of Cu as a micronutrient in plants and microbes.

Table 1.

Growth of *Rhizobium* sp. strain M_1 in liquid culture medium in presence of different concentrations of heavy metal salts, indicated by optical density (A_{560}, 1 cm cuvette) of 4 days old cultures.

ppm	0	25	50	75	100
$NiCl_2$	1.25	0.35*	0.02*	0.01*	0.015*
$Pb(NO_3)_2$	1.15	1.00	0.90*	0,95*	0,85*
$CdSO_4$	1.25	0.02*	0.01*	0.02*	0.03*
$CuSO_4$	1.25	1.05	1.10	1.00	0.35*

* = Significantly different from the control at 1% level.

All the heavy metal salts tested decreased the parameters e. g. number of plants nodulated and number of nodules per plant (Fig. 1), number of nodules per g fresh weight of root (Table 2), nodule dry weight per plant (Fig. 2) and weight per nodule (Table 2). Interestingly, however, the decrease in the number of nodules per plant caused an increase in the nodule dry weight, except in case of $CuSO_4$ and $Pb(NO_3)_2$ at 100 ppm concentration (Table 2). Possibly, as the number of nodules per plant decreased, there was higher mobilization of the reserves to the remaining nodules, leading to an increase in the nodule dry weight. The decrease in the number of nodules per plant caused by these heavy metal salts may be due to the deleterious

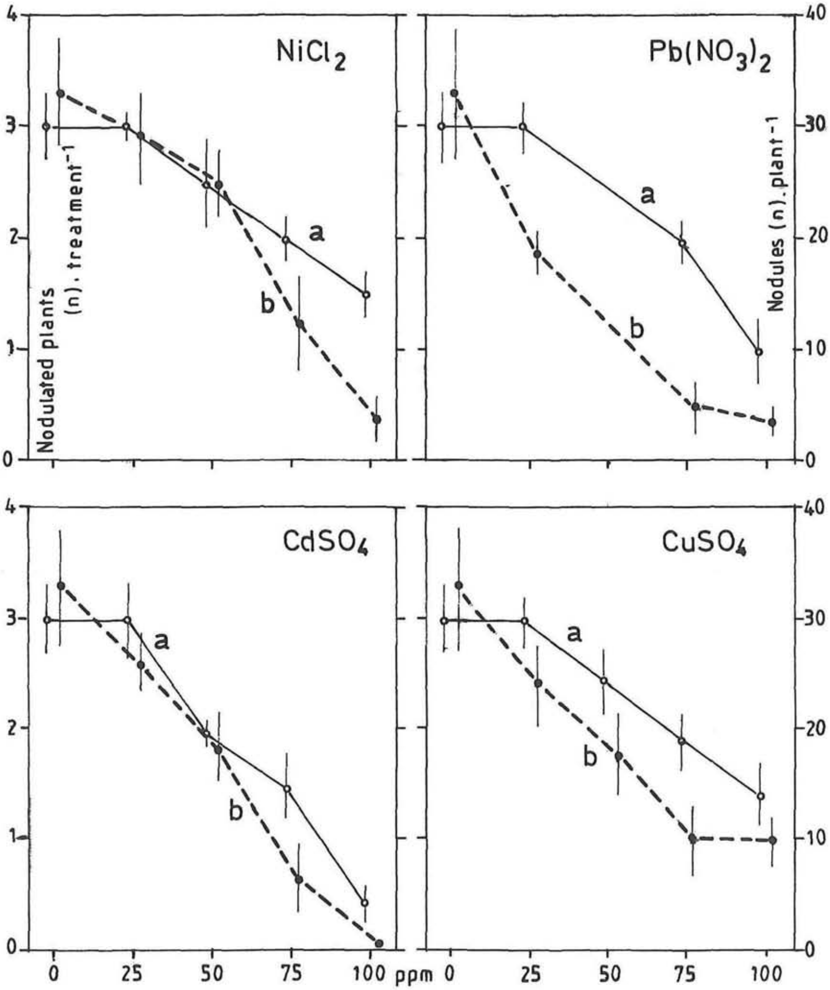

Fig. 1. Effect of different concentrations of NiCl, $Pb(NO_3)_2$, $CdSO_4$ and $CuSO_4$ on the number of nodulated plants (a, scales on the left side) and on the number of nodules per plant (b, scales on the right side). The perpendicular bars indicate the respective standard deviations.

effect of these metals on the growth of *Rhizobium.* Alternatively as the root growth was inhibited by these heavy metals (data not included), fewer sites were available for the infection process. It may be possible that both of these factors together cause an inhibition of the nodulation process. In addition, effect of copper and cadmium oxides in clover plant was due to the effect on the infection process itself rather than on the growth of the bacterium (Yakoleva 1984). Other studies of heave metal interference in nodulation

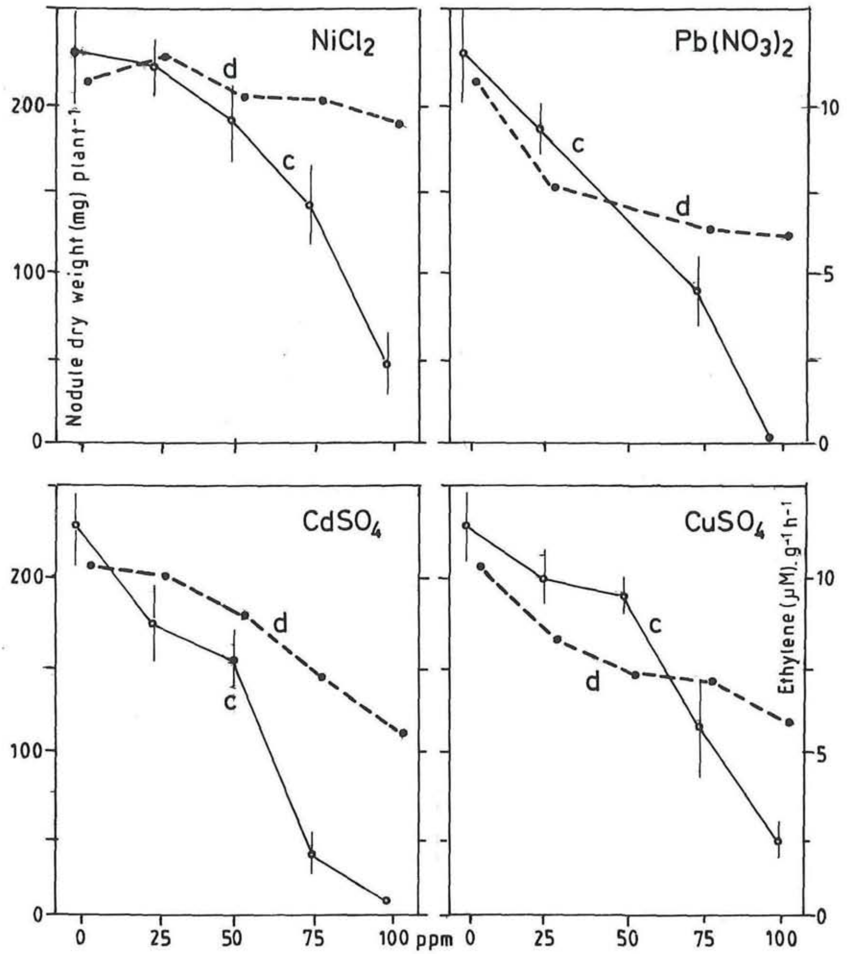

Fig. 2. Nodule dry weight per plant (c, scales on the left side), and acetylene reduction activity (d, black circles, bold broken lines, scales on the right side) as affected by different concentrations of heavy metals. Bars see legend to Fig. 1.

process, however, do not show the mode of heavy metal inhibition in this process (HUANG & al. 1974, MCILVEEN & COLE 1974, PAIVOKE 1983 a, b).

The heavy metal salts, used in this study, except $NiCl_2$ also decreased the acetylene reduction activity of the nodules (Fig. 2). Inhibition of the acetylene reduction activity with heavy metal salt treatment has been reported in several legume *Rhizobium* symbioses (HUANG & al. 1974, MCILVEEN & COLE 1974, PAIVOKE 1983 a b, YAKOLEVA 1984). The mechanism of the inhibition of acetylene reduction activity is not clear and possibly involves several factors like direct interference with the enzyme protein and

Table 2

Effect von heavy metal salts on number of nodules per g f wt of roots (A) and dry weight of nodule (mg, B).

	A: Number of nodules					B: Dry weight of nodule				
ppm	0	25	50	75	100	0	25	50	75	100
$NiCl_2$	47	35.0*	27.6*	16.9*	6.2*	6.3	7.2	7.1	11.7*	5.6
$Pb(NO_3)_2$	47	31.6*	–	5,4*	1.9*	6.3	9.1*	11.3	16.1*	2.7*
$CdSO_4$	47	29.8*	27.3*	4.7*	0.4*	6.3	6.5	7.7	7.1	5.0*
$CuSO_4$	47	40.2	32.6*	14.9*	13.5*	6.3	7.9*	10.2*	8.0*	4.3*

* = differ significantly from control at 1% level.

lower availability of the photosynthate. Heavy metals are known to inhibit photosynthesis (see WOOLHOUSE 1983). Our studies as well as the available reports thus clearly demonstrate that legume-*Rhizobium* symbiosis is highly sensitive to heavy metal toxicity. With regard to the exceptional position of nickel, it seems worthy to note that nickel has proved to be an essential element in Fabaceae being a component of urease, an enzyme involved in the mobilization of nitrogen storage substances in legume seeds (HORAK 1985 a, b). Further studies are required to evaluate the existing variability in terms of metal toxicity for this process as well as to develop heavy metal tolerance for symbiotic nitrogen fixation.

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