Response of *Azotobacter* to Molybdenum Addition to Soil

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ABSTRACT

The population of *Azotobacter* in the soil increased sharply upon addition of simple organic compounds such as sucrose at a rate of 1%. The most-probable number of cells per gram of soil was determined as 5.4×10^7 after three weeks of incubation in both molybdenum amended and non-amended samples compared to 3.3×10^2 in the control. Although addition of molybdenum appeared to have no significant effect on the numbers of *Azotobacter*, its activity in terms of nitrogen fixation was highly influenced by the availability of this element in the soil. The percent total nitrogen increased from 0.034 in the control to 0.063 in sucrose amended soil and reached 0.068 in samples receiving both sucrose and molybdenum. In terms of milligrams of nitrogen fixed per gram of sucrose metabolized, an increase of 2 to 5 milligrams of nitrogen fixed were observed by addition of molybdenum at a rate of 0.1 to 5.0 ppm.

INTRODUCTION

Molybdenum has long been recognized as an essential micronutrient for *Azotobacter* (2). Its biological significance to this free-living nitrogen-fixing bacterium was demonstrated (3, 6). Being an activator of one of the two major proteins that make-up the enzyme nitrogenase renders it indispensable for the nitrogen fixing ability of *Azotobacter*, and the bacterium, in the absence of an adequate supply of the element will grow only when supplied with reduced nitrogen compounds.

Although the amount of this trace element required by the bacterium is very small (6, 7, 8), certain soil conditions have been found to bear some relation to its deficiency (7, 8). Supplementary addition of small amounts of molybdenum to soil was found to enable symbiotic nitrogen fixation but little is known of the effect of added molybdenum on the free-living nitrogen fixers (8).

Results obtained for several soils in El-Jamahiriya show that the amount of molybdenum range from less than 0.01 ppm to slightly more than 0.05ppm (4, 5). The objective of this work was mainly to study the influence of molybdenum addition at different rates on the population and the nitrogen fixing activity of *Azotobacter* in soil.

MATERIALS AND METHODS

A soil sample from a non-cultivated area in Tajura (20 km east of Tripoli) was collected, air-dried and seived to pass a 2-mm screen. Preliminary analysis was made and the data obtained are listed in Table 1. Duplicate subsamples, 100 gram each (oven-dry basis) were weighed in pre-cleaned, oven sterilized 150 ml beakers. All samples received the following: 1.0 gr. sucrose, 0.1 gr. K_2HPO_4, 0.01gr. $MgSO_4.7H_2O$ and 19.0 ml distilled water (Corning AG-3 Glass Distiller). Molybdenum was added as $Na_2MoO_2.4H_2O$ at a rate of 8, 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0ppm. The beakers were then covered with a parafilm and incubated at 28 C for three weeks. At the end of the incubation period, determinations of the percent total nitrogen by steam distillation and the population count of *Azotobacter* applying the most-probable number technique (9), on sucrose-mineral-salt medium were made. (1).

Table 1 — Characterisitics of the soil sample.

Location	Texture	pH	EC mmhos/ cm at 25C	Organic matter (%)	Total nitrogen (%)	Azotobacter count × 10^2	Total bacteria × 10^5
Tajura	Sandy	7.9	0.23	0.64	0.034	3.3	5.2

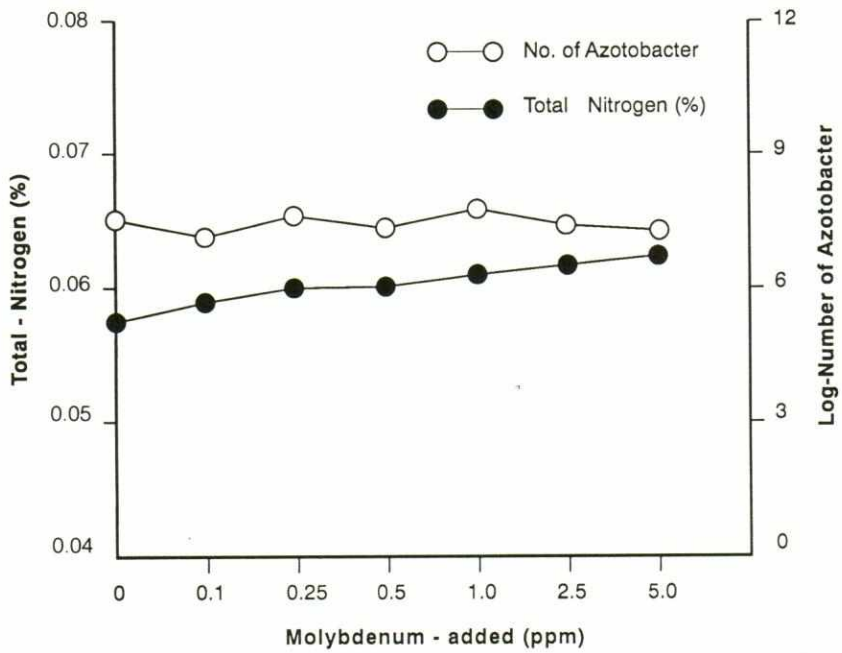

Fig. 1 Effect of added molybdenum on the population count of *Azotobacter* and the percent total nitrogen in soil amended with 1% sucrose. (a) 95% confidence interval was D/3.3 to 3.3 D, where D is the number of cells.

Using the same soil, a second experiment was performed in which three sets of duplicate subsamples (100 gr. each) received 0.1gr. K_2HPO_4, 0.01gr. $MgSO_4.7H_2O$ and 19.0ml of glass-distilled water. Sucrose was added at a rate of 1% to one set, while the second set received, besides sucrose, 5.0 ppm molybdenum as $Na_2M0O_2.4H_2O$. A third set with no sucrose or molybdenum was included as a control. Determinations of the percent total nitrogen and the number of cells of *Azotobacter* were made after 4, 7, 14, 21, 28, and 35 days of incubation at 28C, as described earlier.

RESULTS AND DISCUSSION

From the percent total nitrogen and the log number of cells of *Azotobacter* in the soil at different rates of molybdenum addition shown in Fig. 1, it is evident that the number of cells reflects no significant differences due to the treatments. On the other hand, an increase in the percent total nitrogen was observed with increasing rate of molybdenum added, and if the activity of the *Azotobacter* is expressed in terms of milligrams of nitrogen fixed per gram of sucrose metabolized, an additional 2 to 5 mg. of nitrogen fixing potential were obtained by introducing molybdenum to the soil at a rate of 0.1 to 5.0 ppm (Table 2). This reflects the role this element plays in the activity of the bacterium, and as long as an oxidizable energy source is provided, the rate limiting factor associated with the activity of *Azotobacter* in nitrogen fixation is controlled by the amount of assimilable molybdenum available in the soil.

Table 2 — Effect of molybdenum added at different rates on the amount of nitrogen fixed in a soil amended with 1% sucrose.

Molybdenum added (ppm)	mg-N_2 fixed/gm of sucrose*	Amount of increase in N_2-fixed (mg)
0.00	24	--
0.10	26	2.0
0.25	27	3.0
0.50	27	3.0
1.00	28	4.0
2.50	28	4.0
5.00	29	5.0

* Average of duplicate samples

The results obtained in the second experiment where molybdenum was added at a rate of 5.0 ppm and estimation of the *Azotobacter* population and the percent total nitrogen were made at intervals over a period of 35 days of incubation are presented in Figs. 2 and 3. The number of cells (Fig. 2) increased sharply in the samples to which sucrose (1%) was added in both molybdenum amended and non-amended samples and reached its peak (5.4×10^7 cells/ gr. of soil) after three weeks of incubation compared to the control (3.3×10^2 cells/gr. of soil). This would be expected, knowing that this chemoheterotrophic soil bacterium is classified from the ecological point of view as an indigenous, fast-growing soil organism that respond quickly to simple, water-soluble organic carbon amendments. Although addition of molybdenum appeared to have no significant effect on the total number of *Azotobacter* (Fig. 2), their activity in terms of nitrogen fixation (Fig. 3) is greatly influenced by the presence of this element

in the soil where the percent total nitrogen increased from 0.034 in the control sample to 0.063 in samples amended with sucrose only, and reached 0.068 in samples receiving both sucrose and molybdenum.

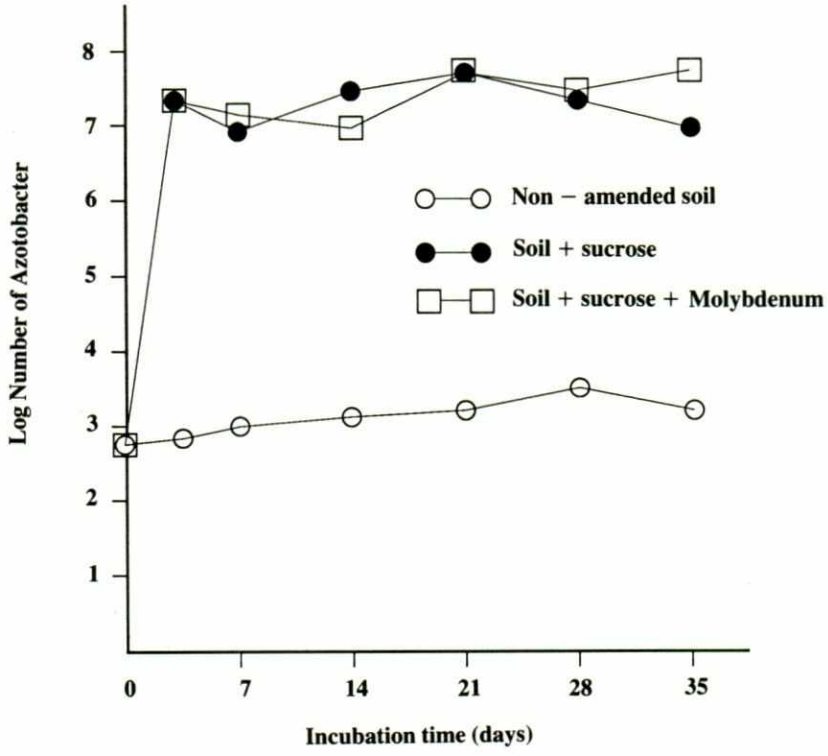

Fig. 2 Estimated most-probable number of *Azotobacter* in soil as influenced by sucrose addition (1%) with and without molybdenum (5ppm). (b) 95% confidence interval was D/ 3.3 to 3.3 D, where D is the number of cells.

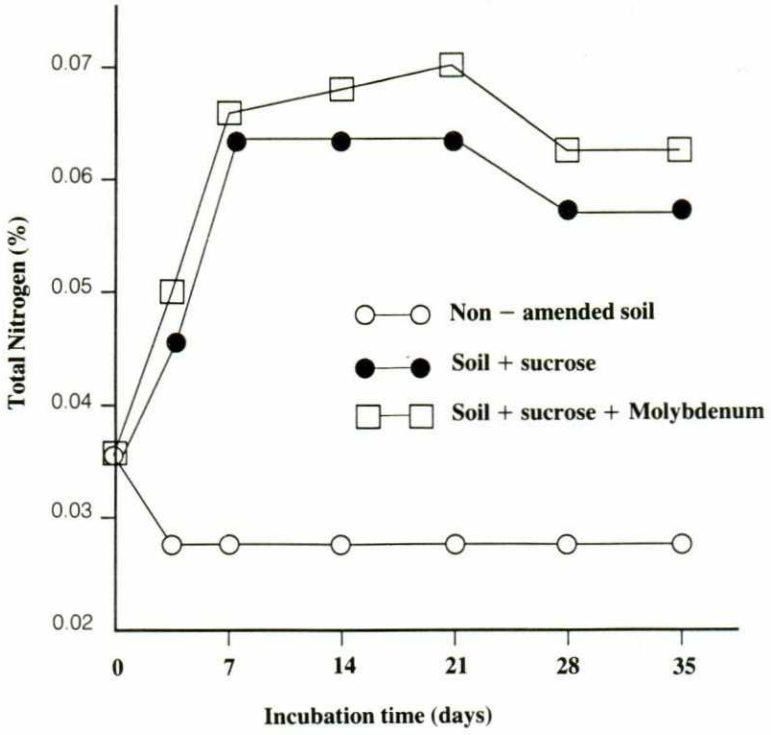

Fig. 3 The percent total nitrogen in soil as influenced by sucrose addition (1%) with and without molybdenum (5ppm).

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استجابة بكتيريا الازوتوباكتر لإضافة الموليبدينوم الى التربة

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المستخلص

زادت أعداد بكتيريا الآزوتوباكتر في التربة زيادة مضطردة عند إضافة مواد عضوية بسيطة التركيب مثل السكروز بنسبة 1%. من نتائج حساب العدد الاحتمالي للخلايا في الجرام الواحد من التربة، وجد أن العدد قد ارتفع الى $10^7 \times 5.6$ من أصل $10^2 \times 3.3$ خلال ثلاثة أسابيع من التحضين.

على الرغم من أن اضافة الموليبدينوم لم تظهر أي فروق معنوية بالنسبة لزيادة أعداد الأزوتوباكتر، إلا أن نشاط هذه البكتيريا في تثبيت النتروجين تأثر بدرجة كبيرة بتوفر هذا العنصر في التربة، حيث ارتفعت النسبة المئوية للنتروجين الكلي في التربة من 0.034 في العينة غير المعاملة الى 0.063 في العينة المضاف إليها السكروز وبلغت 0.068 في العينة المضاف اليها السكروز والموليبدينوم معاً من حساب كمية النتروجين المثبت بالمليجرام لكل جرام في السكروز المستهلك، وجد أن هناك زيادة بمقدار 2.0 الى 5.0 مليجرام من النتروجين المثبت عند إضافة الموليبدينوم بمعدل 0.1 الى 5.0 جزء بالمليون.