The ability of some fungi to utilize minute traces of some heavy metals has been taken advantage of in bio-assay of these metals in soils, plant materials, chemical compounds, culture media, etc. (Mulder, 1939; Steinberg, 1945; Nicholas and Fielding, 1947, 1949, 1950, 1951; Nicholas, 1950; Hewitt and Hallas, 1951; Donald, Passey and Swaby, 1952a and b). The sensitivity of these organisms has, however, varied; but in most cases where they have been used, the accuracy of the quantitative estimation of the elements has been greater than the results obtained by chemical analysis (Nicholas and Fielding, 1951; Hewitt and Hallas, 1951).

In this laboratory the purity of the culture medium to be used in trace element essentiality studies has been tested biologically, using the standard 'M' strain of Aspergillus niger. Simultaneously, a strain of the same fungus, isolated in this laboratory, was compared with the standard strain, as regards its sensitivity. The striking contrast shown by these two strains in their response to deficiencies of some heavy metals is presented.

**Materials and Methods**

*Strains used*

(i) *Aspergillus niger* van Tiegh. 'M' strain kindly supplied by Dr. D. J. D. Nicholas, Long Ashton Research Station, University of Bristol; (ii) *Aspergillus niger* van Tiegh. 'M.U.B.L. 1' (Madras University Botany Laboratory, 1), a strain isolated in this laboratory from the rhizosphere of *Cajanus cajan*, identified and kindly furnished by Mr. V. Agnihothrudu. The cultures were maintained on 2% potato-sucrose-agar.

*Glassware*

Only Pyrex glassware was used throughout. Culture flasks were 500 ml conical, Erlenmeyer type and were covered by 100 ml squat beakers inverted over the open necks. The plugging was done by packing an annular ring of cotton in the space between the beaker and the neck below the rim and...
all glassware used were thoroughly cleaned and dried before each experiment (Hewitt and Hallas, 1951). Final rinsing was done in water distilled thrice over in Pyrex all-glass stills.

**Distilled water**

Thrice Pyrex glass distilled water was used in the preparation of media as well as micro-nutrient solutions.

**Basal medium**

A sucrose-nitrate medium (derived from high quality analytical reagents) of the following formula was used:

\[
\begin{align*}
KNO_3 & : \quad 10 \text{ g.} \\
KH_2PO_4 & : \quad 5 \text{ g.} \\
MgSO_4 \cdot 7H_2O & : \quad 2.5 \text{ g.} \\
\text{Sucrose} & : \quad 50 \text{ g.} \\
\text{Distilled water} & : \quad 1 \text{ litre}
\end{align*}
\]

**Purification of basal medium**

The Al₂O₃ (B.D.H. Chromatographic analysis material) method devised by Donald, Passey and Swaby (1952b) was adopted to study deficiencies of Fe, Zn, Cu and Mn, and the H₂S co-precipitation method advocated by Hewitt and Hallas (1951) for Mo deficiency studies, double purification having been carried out in the latter case. All filtrations were done using acid-washed No. 42 Whatman* filter-paper. The pH of the purified medium was adjusted to 5.0. 25 ml. aliquots were apportioned in the culture flasks, covered and sterilized at 15 lb. pressure for 15 minutes.

**Micronutrient solutions**

Six micro-nutrient stock solutions were prepared: a complete solution, with all the five heavy metals studied, (+ all) and five others in which Fe, Zn, Mn, Cu and Mo were omitted singly for the respective deficient cultures (— Fe, — Zn, — Mn, — Cu and — Mo series). The micro-nutrient solutions were added to each flask to give the following concentrations per 25 ml. of medium.

\[
\begin{align*}
\text{FeCl}_3 \cdot 6H_2O & : \quad 75 \mu\text{g.} \\
\text{ZnSO}_4 \cdot 7H_2O & : \quad 50 \mu\text{g.} \\
\text{CuSO}_4 \cdot 5H_2O & : \quad 15 \mu\text{g.} \\
\text{MnSO}_4 \cdot 4H_2O & : \quad 7.5 \mu\text{g.} \\
(NH_4)_6\text{Mo}_7\text{O}_{24} \cdot 4H_2O & : \quad 10 \mu\text{g.}
\end{align*}
\]

Merck's guaranteed Reagent grade