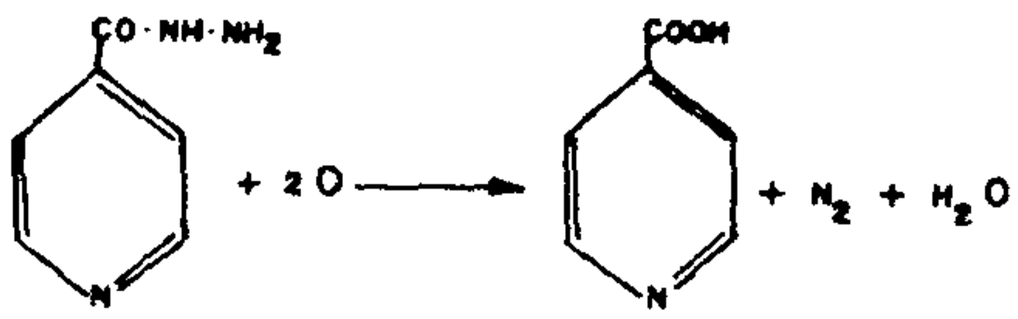
10. Besson, E., Chopin, J., Gunasegaran, R. and Nair, A. G. R., *Phytochem.*, 1980, **19**, 2787.
11. Bouillant, M. L., Arce, F. F., Bonvin, J. F., Chopin, J., Zoll, A. and Mathieu, G., *Phytochem.*, 1979, **18**, 1043.

A NEW METHOD FOR THE ESTIMATION OF ISONIAZID

A. MURUGESAN AND D. VENKAPPAYYA
Department of Chemistry, Regional Engineering College, Tiruchirapalli 620 015, India.

ISONIAZID, isonicotinohydrazide, is a highly effective tuberculostatic drug and many methods[1-7] are available for its determination. We report here yet another easy and reliable method using the reducing property of isoniazid. We have tried as oxidant, potassium permanganate, which oxidises isoniazid to isonicotinic acid. The oxidation of isoniazid by permanganate is somewhat slow at room temperature and the reaction is complete within 5 min at a temperature of 50–55°C. The equivalent weight of isoniazid for this reaction is established as molecular weight divided by four as shown below:



A definite quantity of the aqueous isoniazid solution is treated with a known excess of the $KMnO_4$ solution. The unreacted $KMnO_4$ is determined by adding pottassium iodide solution and titrating the liberated iodine against a standard sodium thiosulphate solution.

Isoniazid powder (0.75 g) is dissolved in 30–50 ml of water. In the case of the tablets, the solution is filtered to a 250 ml standard flask and made up by repeatedly washing with water. The isoniazid solution (20 ml) is pipetted into an iodine flask, 25 ml of 0.1 N $KMnO_4$ and 20 ml of 2N H_2SO_4 are added and heated for 5 min at 50-55° C and allowed to cool to room temperature. To this solution 20 ml of 10% KI is added and the liberated idodine is titrated against standard thiosulphate solution using starch as indicator (V_1). The end point is the discharge of blue colour. By a separate experiment the thiosulphate, eqvivalent of 25 ml of $KMnO_4$ solution (V) is determined.

$$\text{Percentage of isoniazid} = \frac{(V_2 - V_1) \times S_{thio} \times 34.27 \times 100}{20 \times 4 \times W}$$

where W = weight of isoniazid powder taken.

This method can be used to estimate pure isoniazid as well as ioniazid in various tablet forms such as Dosina. The error is found to be less than ±0.5%.

3 November 1982

1. Scott, P. G. W., *J. Pharm. Parmacol.*, 1952, **4**, 68.
2. Kuhni, E., Jacob, M. and Grossglauser, H., *Pharm. Acta. Meko.*, 1954, **29**, 233.
3. Budesinsky, B., *Ceskosl. Farm.*, 1955, **4**, 185.
4. Nayak, R. N., Yathirajan, M. S. and Manjappa, S., *Curr. Sci.*, 1981, **50**, 812.
5. Ramanna Rao, G., Benerjee, S. K. and Ramamohan, *Indian J. Pharm. Sci.*, 1981, **43**, 154.
6. Yathirajan, M. S., Rangaswamy and Mahadevappa, D. S., *J. Indian Chem. Soc.*, 1981, **58**, 619.
7. Garratt, D. C., *The quantitative analysis of drugs*, III Edition, p. 360, Chapman and Hall Ltd.

COPPER INTERFERENCE IN NITRITE ESTIMATION BY DIAZOTIZATION AND ITS ELIMINATION

C. SUBRAMANYAM AND G. VENKATESWERLU
Department of Biochemistry, Osmania University, Hyderabad 500 007, India.

NITRATE and nitrite are two important anions occurring in water, soil, vegetables and industrial waste whose concentrations need a careful evaluation. In many cases the concentration of nitrite is determined by a colorimetric procedure using a diazotization reaction[1,2]. This method is adapted for the determination of nitrate also after its reduction to nitrite. Automated systems have been developed for the rapid determination of these ions[3]. In such systems metallic wire reductors, treated with copper sulphate solutions have been employed to convert the nitrate into nitrite where contamination of the sample with copper is possible. Copper catalyses the decomposition of the diazonium salt and yields low results[2]. This was clearly observed when we attempted to determine the accumulation of nitrite in the culture medium of *Neurospora crassa* under conditions of copper toxicity[4].

In order to obviate this problem, copper was eliminated as its sulphide and the nitrite estimated by developing colour with sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride[5]. The experimental details and the results are discussed below.

Neurospora crassa was grown for 72 hr on 10 ml basal medium containing sodium nitrate (0.52%) as nitrogen source and graded amounts of copper sulphate[4]. At the end of the growth period, the medium was quantitatively collected and the nitrite content determined.

Determination of nitrite: Samples (0.5 ml) containing nitrite (upto 8 μg) were made upto 2 ml with water in a test tube to which 50% ammonium chloride (0.2 ml) was added followed by ammonia (0.1 ml, Sp.Gr. 0.91). Hydrogen sulphide was passed with constant mixing for 15 minutes to precipitate copper. The tubes were allowed to stand for an hour and the precipitate removed by centrifugation. Excess hydrogen sulphide was eliminated (lead acetate test) by keeping the tubes in a boiling water bath. To the samples, free of hydrogen sulphide, 1% sulphanilamide (0.5 ml) was added followed by 0.005% N-(1-naphthyl)-ethylenediamine dihydrochloride (0.5 ml)[6]. The total volume was made up to 10 ml and the absorbance of the coloured solutions measured at 540 nm.

The standard calibration curve for nitrite estimation before and after treating the samples with hydrogen sulphide to eliminate copper is depicted in figure 1. From this data, it is evident that hydrogen sulphide completely eliminates the interference due to copper. Absorbance obtained after the elimination of copper is in close agreement with values obtained with copper-free samples. The efficiency of the procedure indicated in table 1 shows the data to be accurate up to 99.8% provided copper is eliminated meticulosuly.

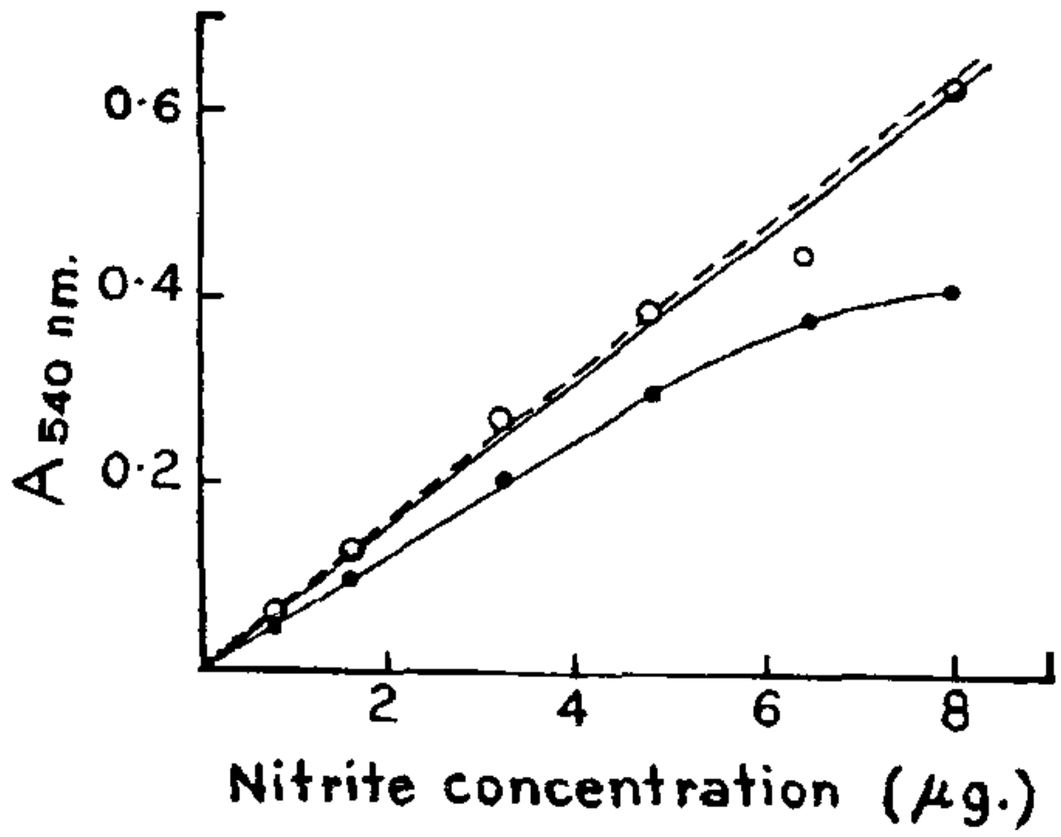


Figure 1. Interference by copper in nitrite estimation by diazotization. Open circles and closed circles in solid line indicate respectively absorbance in the absence of and presence of copper. The dashed line denotes absorbance after copper elimination.

9 September 1982; Revised 10 December 1982

1. Snell, F. D. and Snell, E., In *Colorimetric methods of analysis*, Van Nostrand Reinhold, New York, Vol. 2, 1949.

TABLE 1

Recoveries of the added nitrite to the medium

Copper content in the medium (μg)	NO_2^- content in medium after elimination of copper (μg)	NO_2^- content in medium after addition of 3.2 μg of NO_2^- (μg)	Observed NO_2^- after deducting NO_2^- found in medium (μg)	Recovery (%)
Nil	Nil	3.2	3.2	—
200	2.4	5.6	3.2	100
400	2 05	5.2	3.15	98.4
600	1.06	4.3	3.24	101

Medium (0.5 ml) was taken from *N. crassa* cultures after 72 hr of growth and nitrite was assayed after treating with hydrogen sulphide. Where necessary, nitrite was added and the total nitrite was analysed after elimination of copper. Values represent average of three experiments.

2. Taras, M. J., In *Colorimetric determination of non-metals*, 1958, Interscience, New York, p. 75.
3. Willis, R. E., *Anal. Chem.*, 1980, **52,** 1376.
4. Venkateswerlu, G. and Sivarama Sastry, K., *Indian J. Biochem. Biophys.*, 1979, **16,** 84.
5. Bratton, A. C., Marshall, E. K., Babbitt, D. and Hendrickson, A. R., *J. Biol. Chem.*, 1939, **128,** 537.
6. Nason, A. and Evans, H. J., *Methods Enzymol.*, 1955, **2,** 411.

ADDITIONAL FAUNAL MATERIAL FROM THE PLEISTOCENE FORMATION OF THE RIVER GHOD-A TRIBUTARY OF THE BHIMA, MAHARASHTRA, INDIA.

G. L. BADAM, R. K. GANJOO AND SALAHUDDIN
Deccan College, P. G. and Research Institute, Pune 411 006, India.

THE Ghod is an easterly flowing tributary of the river Bhima originating in the Western Ghats, about 75 Km northwest of Pune, and flowing for a distance of about 160 km over the Deccan Traps before its confluene with the river Bhima. While the only notable occurrence of fossil vertebrates from the Bhima comes from Hagargundi[1], a Middle Palaeolithic site, the Ghod river has in recent years yielded half a dozen palaeontological sites namely Inamgaon, Sirasgaon Kanta, Chinchini, Chandoli, Khadki and Kalamb[2] (see figure 1). All the sites are located upstream of Inamgaon–the locality that has yielded one of the richest palaeontological treasures in the Deccan.

The Pleistocene formations in the Ghod Valley are fluvial in origin and rest unconformably on the Deccan Traps of Cretaceous-Eocene age. Two litho-units can generally be identified, the basal sandy pebbly gravel about 5 m thick capped by a 5 to 10 m thick unit of fine sands, silts and clays which in turn is overlain about a meter thick Black Cotton Soil of early Holocene age.

The species identified so far, out of a collection of about 200 fossil fragments, include osteological and dental remains belonging to *Elephas maximus, Elephas hysudricus, Bos namadicus, Bubalus* sp., *Cervus unicolor, Canis* sp., *Equus namadicus, Hippopotamus* sp. and Chelonia (possibly *Trionyx*)[3]. The presence of fossil hippo in the Ghod is of special significance as it increases the geographical range of this animal to the south of Godavari before becoming extinct and has a profound bearing on the palaeoecology of the Ghod at a time when the sediments were being deposited. Two lower molars (M_2 and M_3) of this animal were found in a gravel bed associated with molluscan shells (*Unio*) which have been C-14 dated to 20,000 years B.P.[4]. Majority of the other fossils come from the base of the yellow silt which is strongly calcreted. Excavations carried out during the last three years in these formations, have yielded Middle Palaeolithic tools (chiefly scrapers) along with fossils

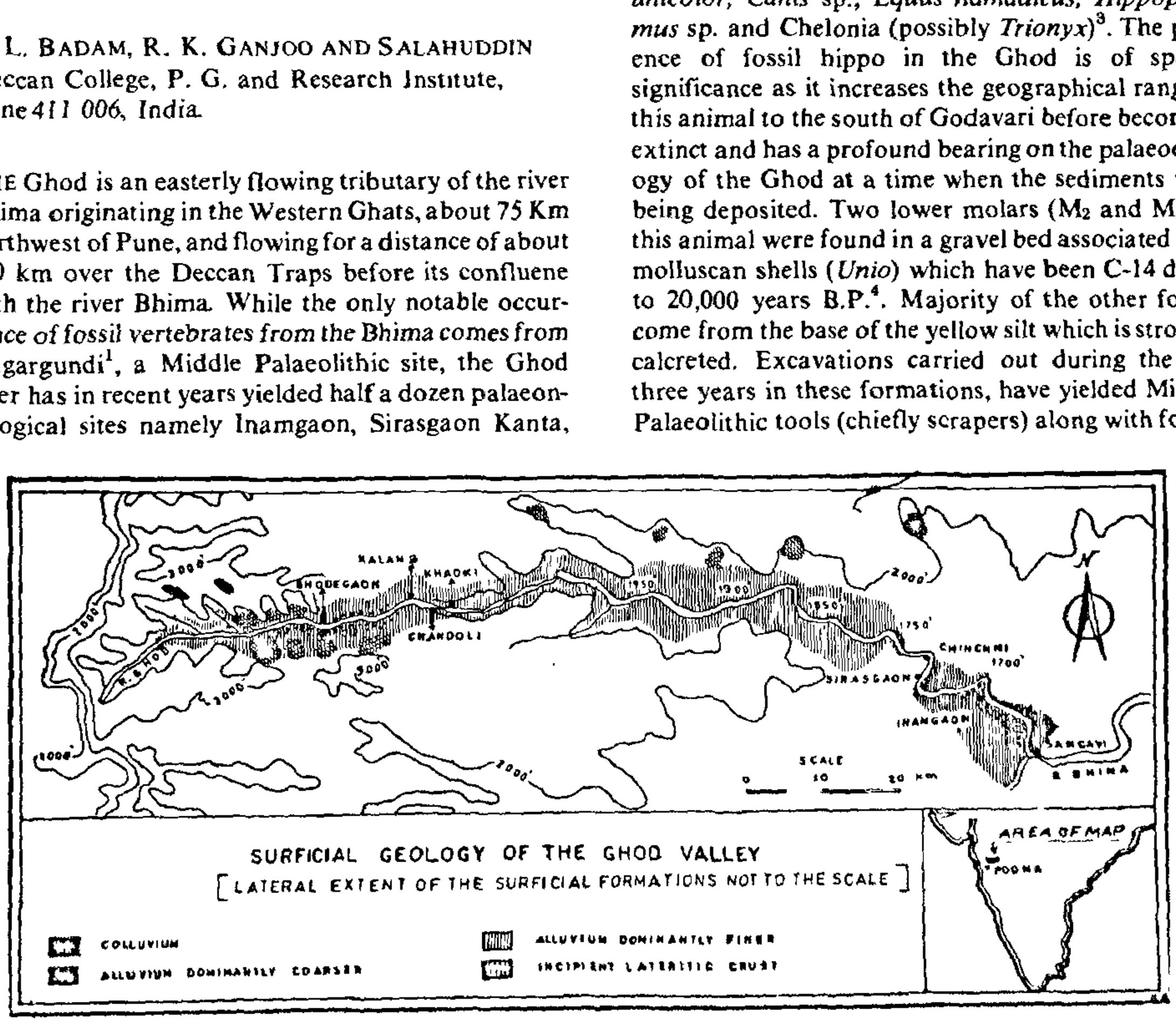


Figure 1. Fossil localities on the Ghod river.