POT SAND CULTURE TECHNIQUE FOR THE STUDY OF MINERAL NUTRIENT ELEMENT DEFICIENCIES UNDER INDIAN CONDITIONS

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ABSTRACT

Paper describes the suitability of silica sand obtained from upper Kaimur sandstone beds south-west of Allahabad (U. P.) for refined sand culture work on plant nutrition. The procedures for the purification of sand, water and culture nutrients, and the suitability of the different types of vessels for controlled culture of plants are described.

Extensive culture trials in which a wide variety of plants were grown at normal and deficient levels of particular macro- and microntrient elements confirmed that the sand culture technique as developed at the Botany Department, Lucknow University and described in the paper, can be suitably employed for investigating macro- and micronutrient problems of plants under controlled conditions. All the requirements of the technique, except a special (Kee-bush) sand digestor essential for purifying sand for growing plants at known low levels of copper, zinc and molybdenum, can be met indigenously.

INTRODUCTION

It is well known that refined pot sand culture studies provide information which is of great value in elucidating the role of minieral nutrients in plants and problems of nutrient availability in soils. HEWITT (1952) has given a comprehensive account of the sand culture techniques used in the study of plant nutrition in England and many other western countries. Such studies have, however, not received much attention in India so far. A review of literature showed that upto 1955, when studies described in this paper were first under taken, the few workers in India who had grown plants in sand culture for macronutrient element studies had used river bed sand (SIRCAR & SEN, 1941; MISRA & SAMANT RAI, 1955). GANDHI AND MEHTA (1959) and NEELKANTAN AND MEHTA (1961) made use of river bed sand after cold hydrochloric acid treatment even for studies on micronutrients. Since the size of the particle and the chemical composition of the river bed sand renders it unfit for refined sand culture work, SARIN AND SAXENA (1963) and PRAKASH AND SAXENA (1964) made use of purified quartz sand for raising seedlings which were subsequently transferred to culture solutions. While quartz sand is very suitable for refined sand culture work its availability and cost prohibits its use for large scale pot culture studies.

In order to undertake large scale pot sand culture of plants, it seemed necessary to locate an indigenous source of sand from which could be drawn, year after year, sand of suitable quality which could be purified with ease and to test some other points of technique, e.g. how far the ion exchange columns, particularly the one available in India, could be economically used for purifying the town water supply for obtaining acute effects of macro and micronutrient element deficiencies and whether the clay, ploythene, or glass containers available locally could be used for work on macro and micronutrients in plants. In an earlier publication (AGARWALA AND SHARMA, 1961) we gave a preli-

minary account of the procedure worked out for the purification of sand obtained from sandstone deposits in U.P., which is widely used for the manufacture of glass in India, and of certain other points of culture technique developed by us at the Botany Department of the Lucknow University. This paper describes the details of the large scale sand culture technique which has since been found suitable for investigating macro and mironutrient element problems of a wide variety of plants.

EXPERIMENTAL

Since it was desired to develop the purification methods which could be adopted with ease, were least cumbersome and inexpensive, several variations in the essential requirements of the sand culture technique as described by HEWITT (1952) were tried. A particular constituent or a purification procedure was considered suitable when the desired extent of the deficiency of the macro or micronutrient elements was obtained by using it.

The composition of the nutrient solution used in the initial trials was essentially the same as used at the Research Station, Long Ashton, Bristol, U. K. (HEWITT, 1952). The macro- and micronutrient elements were supplied as under :

4 mM Ca $(NO_3)_2$; 4mM KNO_3; 2mM $MgSO_4$ $7H_2O$; 1.33 mM NaH_2PO_4;
0.1 mM $FeC_6H_5O_2$ H_2O; 10 mM Mn SO_4 $4H_2O$; 1 μM $CuSO_4$ $5H_2O$;
1 μM $ZnSO_4$ 7 H_2O; 33 mM H_3BO_3; 0.2 μM Na_2MoO_4 $2H_2O$;
0.1 μM $CoSO_4$ $7H_2O$; and 0.1 μM $NiSO_4$ $7H_2O$.

For obtaining macronutrient deficiencies the element under study was fully or partly omitted. When calcium, magnesium and potassium were omitted an equivalent amount of sodium was substituted; when nitrogen was omitted calcium and potassium were supplied as sulphates; when phosphorus was omitted sodium sulphate was substituted in place of sodium phosphate; when sulphur was omitted magnesium was supplied as chloride instead of sulphate. For micronutrient deficiencies the element under study was fully omitted.

TESTING THE PURITY OF SAND, WATER AND NUTRIENT SOLUTIONS

Macro and micronutrient content was determined in sand at various stages of purification, in water, in the culture nutrients obtained from different sources and purified in different ways, and in the experimental plants.

Sand—Sand was first water washed and then treated with 20% hydrochloric acid in Soxhlet apparatus for 8 hours. The hydrochloric acid extract was analysed for different macro and micronutrient elements.

Water—Suitable volume—5 to 10 L—of water was evaporated in pyrex erlenmeyer flasks. The residue in the flasks was dissolved in a minimum quantity of A. R. grade redistilled hydrochloric acid and analysed for the different macro and micronutrient elements.

Nutrient solution—Aqueous solutions of macronutrient salts manufactured by reputed concerns were estimated for different micronutrients, before purification and after purification by appropriate methods.

Culture vessels—For testing the suitability of different types of containers for a particular nutrient element deficiency, plants receiving (normal and deficient) supply of that element were raised in bitumen painted clay pots, polythene pots and glass pots, and their performance in these containers was compared by determining the dry matter yield and the tissue concentration of the nutrient element concerned. The dry matter

yield of plants was determined by drying the plant material in a forced drought oven at 70°C for 24 hours.

For determining the nutrient status of the normal and deficient plants the estimations of the various macronutrients except nitrogen were carried out on oven dry plant material digested in nitric-perchloric acids according to the method described by PIPER (1942). For estimation of nitrogen oven dry plant material was digested by the method of CHIBNALL, REES AND WILLIAMS (1943).

Sodium, potassium and calcium were determined flame photometrically. Magnesium and phosphorus were determined colorimetrically by the method developed by NICHOLAS (WALLACE, 1951). Sulphur was determined turbidimetrically by the method of CHESNIN & YIEN (1951). nitrogen was estimated by the semi-micro-kjeldahl method (CONWAY & O'MALLEY, 1942). Iron was determined as ferrous-orthophenanthroline complex (HUMPHRIES, 1956). Copper was extracted in dithizone and estimated as copper carbamate by PIPER's modification (1942) of the method of SYLVESTER AND LAMPITT (1940). Manganese was determined either by the 'tetrabase' method of NICHOLAS AND FISHER (1950) or by the formaldoxime method developed by NICHOLAS (WALLACE, 1951.) Molybdenum was estimated as molybdenum—dithiol complex by the method of PIPER AND BECKWITH (1948) and zinc as zinc-dithizonate by the method of COWLING AND MILLER (1941).

OBSERVATIONS AND CONCLUSIONS

SAND AND ITS PURIFICATION

Preliminary work carried out at the Botany Department, Lucknow University, showed that river bed sand from the rivers—Jamuna, Ganges and their tributaries was not suitable for raising plants in culture. The particles of the river bed sand were very fine and were mixed with silt and mica. The removal of these particles was extremely difficult. Besides, fineness of the particles the river bed sand caused water logging and poor aeration in the culture vessels. Sand for raising plants in culture was, therefore, obtained from sandstone deposits. Available information on sand stone beds of India (MISRA, 1942; MISRA & SINGH, 1942) revealed that sand from sandstone beds extending between Jasra and Bargarh, south west of Allahabad (U.P.) is rich in silica, soft and friable and, therefore, largely used in glass Industry as glass-making sands. It is, therefore, easily available. The mechanical and chemical composition of the sand (Table 1) querried from the sand stone beds of the upper Kaimur series south west of Allahabad indicated that the sand from the aforementioned deposits could be, after suitable treatment, successfully utilized for culture work. To confirm this a large number of sand samples were collected from Loghara, Bargarh, Shankergarh and adjoining areas in Allahabad and Banda districts of U.P. The size of the sand grains and their colour varied considerably. The colour of the sand varied from white to deep tints of yellow, reddish yellow, buff and pink. The tinted sand was rich in iron which could be extrcted in hot 20% hydrochloric acid (Table 2). On the basis of the chemical analysis (Table 2), white silica sand seemed to be more suitable for the culture studies than the tinted sand. This was also confirmed by plant growth studied on acid washed white and tinted silica sands.

In a preliminary trial the effect of sand grain size was tested on the growth of a number of plant species. The trial showed that the growth of barley (*Hordeum vulgare*) and gram (*Cicer arietinum*) was more vigorous in the fraction retained between 20 and 60 mesh U. S. standard sieves (particle size 0.25 to 0.84 mm) than in the fraction retained

Table 1—Mechanical and chemical analysis of Bargarh sand* .

Mechanical analysis			
Retained on	Mesh	Percentage	Cumulative percentage
,,	10	0.0	0.0
,,	20	0.0	0.0
,,	30	0.5	0.5
,,	40	1.5	2.0
,,	50	41.0	43.0
,,	60	24.0	67 .3
,,	80	20.0	87.0
,,	90	7.0	94.0
,,	100	4.2	94.2
Passing	100	2.0	100.2

Chemical analysis	
SiO_2	97.69
Al_2O_3	1.72
Fe_2O_3	0.04
CaO	0.28
MgO	Traces
Loss on ignition	0.54
Total	..100.27

*Data provided by the Glass Technologist to the Government of Uttar Pradesh (personal communication).

Table 2—Limits of macro and micronutrient elements which could be extracted from a typical Shankaergarh sand by Soxhlet extraction with 20% hydrochloric acid.

Colour of sand	Limit	Nutrient element									
		K	Ca	Mg	P	S	Fe	Mn	Cu	Zn	Mo
		μg/g. sand									
White	Min. ..	3.9	65	1.0	2.1	1.9	45	0.01	0.06	2.1	0.002
	Max. ..	5.1	98	2.2	3.5	3.5	78	0.14	0.90	3.4	0.003
Tinted	Min. ..	4.1	295	1.0	2.7	1.9	380	0.09	0.8	3.1	0.002
	Max. ..	5.8	8000	3.7	4.1	3.8	1440	17.00	1.3	3.7	0.003

on 20 or passing 60 mesh sieves (particle size>0.84 mm and <0.25 mm). Accordingly, for all culture work sand of the grain size 0.25 to 0.84 mm was used. This was subsequently found to be satisfactory for the growth of a wide variety of plants.

The purification of sand was carried out in (i) wooden vats, (ii) clay pots (*nad*) (iii) high density polythene bins and (iv) 'Kee-bush' sand digestor as described by HEWITT (1947). Prior to use (i) and (ii) were painted on the inside with 'bitolin' brand of bitumen obtained from M/s. British Paints (India) Limited. The purification treatments were given to sand in 50 kg batches in case of (i) to (iii) and in a 150 kg. batch in case of (iv).

As a first step towards purification of sand it was freed of silt, clay, etc. by releasing a stream of running water under pressure at the bottom of sand contained in clay pots painted with bitumen. Water washing of the sand was carried out for 3 to 5 hours before the sand could be freed of silt particles. The water washed sand was further purified by one or more of the following procedures :

Cold hydrochloric acid treatment (Treatment I)—Cold acid treatment was given by saturating the air dierd water washed sand with 4% w/v commercial grade hydrochloric acid. Sand was kept saturated with hydrochloric acid for three days.

Hot hydrochloric acid treatment (Treatment II)—1 L of 17% w/v C.P. grade hydrochloric acid was added per 10 kg sand. The sand-acid mixture was agitated by blowing steam at a pressure of 20 lbs/sq. inch in the acid sand mixture for 4 to 6 hours.

Hot hydrochloric acid-oxalic acid treatment (Treatment III)—This was similar to treatment II but in addition to 1 L of 17% w/v hydrochloric acid, 10 g. oxalic acid/L of hydrochloric acid were also added per 10 kg of sand.

Hot alkali treatment (Treatment IV)—This treatment was given to sand which had already been given treatent III. 1 L of 2% potassium hydroxide was added per 10 kg of acid washed sand after thorough leaching with water. Alkali-sand mixture was agitated as in the case of treatment II by blowing steam for 4 to 6 hours.

The treatment IV was invariably followed by thorough washing with deionised water and the repetition of treatment III.

While treatment I was given only in bitumen painted clay pots, the other treatments were carried out in each of the four types of sand treating vessels (i to iv) described above. The steam required for agitating the sand-acid or the sand-alkali mixture was generated in a 'Bastian and Allen' electrode steam boiler or alternatively in an autoclave heated by a kerosine oil high pressure stove or a gas burner.

After each of the above indicated treatments, the sand was thoroughly washed with water. Final leaching of the sand, prior to use, was carried out with 0.06% calcium nitrate solution, till, on standing overnight, the pH of the sand ranged 6.5 to 7. Where sand was required for work on calcium deficiency, the final leaching was carried out with full nutrient solution omitting calcium. When sand was required for micronutrient deficiency work, calcium nitrate used for leaching was appropriately purified prior to use.

The extent of the purification carried out depended on the purpose for which the sand was required. The sand was considerd pure for a particular macro or micronutrient element deficiency work only when the desired extent of deficiency could be produced in the purified sand where the element in question was excluded from the culture solution. In tables 3 to 5 are given the dry matter yield and normal and deficient values of some mineral nutrient elements in certain crops raised in sand purified by the different treatments. On the basis of the results obtained by culture of a wide variety of plants in sand purified by the different treatments the following conclusions have been drawn regarding the suitability of the sand purification procedure :

Sand purification procedure	Suitability for obtaining
(i) Treatment I	Mild deficiencies of potassium, phosphorus nitrogen.
(ii) Treatment II	Severe deficiency of all the macronutrients and mild deficiency of iron.
(iii) Treatment III	Severe deficiency of iron.
(iv) Treatment III (twice)	Acute deficiency of iron and boron in a wide varetiy of plants and mild manganese dificiency in high manganese requiring crops like the cereals and sugarbeet.
(v) Treatment III (Four times) or Treatments III, IV, III, IV, III in that order.	Acute manganese deficiency and mild to moderate deficiency of copper and zinc.
(vi) as (v) but each treatment given for 6 hours duration in a 'Kee-bush' sand digestor.	Acute deficiency of all the micronutrients including molybdenum.

Table 3—Dry matter yield of five plant species grown at normal and deficient levels of macronutrient elements in sand purified by the different methods.

Plant	Age of plants	Sand purification procedure	Deficiency treatment						
			None	—K	—Ca	—Mg	—P	—S	—N
			g dry matter/plant						
		Water washed ..	4.6	4.0	3.8	4.3	3.9	4.6	3.0
Capsicum ..	9 weeks ..	Treatment I ..	4.8	2.7	2.6	4.9	2.8	4.0	1.6
		Treatment II ..	4.7	1.5	1.8	3.6	1.1	3.6	0.9
		Water washed ..	12.8	10.6	6.7	11.3	9.7	8.8	7.6
Cabbage ..	12 weeks ..	Treatment I ..	12.7	7.8	5.2	8.9	4.3	6.1	3.2
		Treatment II ..	13.6	5.6	3.7	11.2	3.9	3.8	0.2
		Water washed ..	4.9	4.0	3.8	4.8	3.9	4.7	3.9
Okra ..	12 weeks ..	Treatment I ..	5.2	3.7	2.8	5.3	3.1	3.9	1.4
		Treatment II ..	5.1	2.0	1.9	4.5	2.9	3.3	0.3
		Water washed ..	4.7	3.8	3.0	3.9	3.0		1.7
Barley ..	9 weeks ..	Treatment I ..	4.1	2.0	1.8	3.7	1.8		2.3
		Treatment II ..	4.2	2.6	1.4	3.6	1.4		1.2
		Water washed ..	12.8						
Radish ..	8 weeks ..	Treatment I ..	13.1						
		Treatment II ..	12.9						

Table 4—Dry matter yield of some crop plants grown at normal and deficient levels of the micronutrient elements in sand purified by different treatments*.

Nutrient element	Plant	Normal (N) or Deficient (D)	Sand purification treatment				
			II	III	III (twice)	III (Four times)	III, IV, III IV, III (in that order)
			g dry matter/plant				
Iron	Barley	N	2.10	1.80	1.69	1.70	1.68
		D	1.95	1.22	0.31	0.31	0.32
	Cauliflower	N	3.95	3.91	4.10	4.25	4.15
		D	3.10	2.85	1.80	1.96	1.95
	Mangold	N	3.88	3.56	3.85	4.01	3.86
		D	3.95	3.41	2.41	2.11	2.50
Manganese ..	Rice	N	2.10	1.88	1.79	1.81	2.00
		D	1.95	1.65	1.05	0.55	0.60
	Sugarbeet	N	4.11	4.01	3.69	3.65	3.60
		D	3.88	2.96	1.32	0.29	0.30
	Cauliflower	N	3.95	3.67	3.40	3.33	3.40
		D	4.10	3.01	2.70	2.16	2.75
Copper	Wheat	N		2.35	2.19	2.00	2.12
		D		1.65	0.91	0.31	0.39
	Barley	N		2.20	2.10	2.11	2.27
		D		1.81	1.30	0.28	0.29
	Cauliflower	N		3.15	3.40	3.10	3.25
		D		2.99	2.10	1.70	1.88
Zinc	Wheat	N		1.98	1.88	1.90	1.90
		D		1.66	0.61	0.25	0.31
	Maize	N		2.00	2.00	1.85	1.99
		D		1.71	0.41	0.22	0.24
	Gram	N		1.86	1.79	1.80	2.01
		D		1.66	0.95	0.75	0.82
Molybdenum	Maize	N		4.01	3.96	3.85	3.91
		D		3.85	2.60	1.10	0.90
	Cauliflower	N		4.22	4.10	3.85	3.92
		D		3.95	2.10	1.08	0.99
	Radish	N		3.80	3.65	3.45	3.42
		D		3.69	2.57	1.07	0.98
Boron	Wheat	N		2.66	2.54	2.55	2.55
		D		2.00	1.78	1.65	1.59
	Radish	N		1.31	1.28	1.30	1.30
		D		0.69	0.39	0.38	0.37
	Cauliflower	N		3.52	3.41	3.11	3.22
		D		2.38	1.65	1.55	1.50

*Data obtained from different trials. Values pertain to plants at different stages of growth.

Table 5—Normal and deficient values of macronutrient elements in leaves of certain plants grown in sand purified by hot acid treatment (Treatment II).

Plant	Normal (N) or Dedcient (D)	Macronutrient element					
		K	Ca	Mg	P	S	N
		Tissue concentration % dry wt.					
Cabbage	N ..	3.25	2.16	0.29	0.58	1.11	3.17
	D ..	2.25	1.19	0.21	0.11	1.10	1.13
Okra ..	N ..	0.89	4.19	0.52	0.58	0.17	..
	D ..	0.63	1.62	0.47	0.21	0.15	..
Gram ..	N ..	3.30	5.30	0.34	0.43	0.48	2.82
	D ..	0.04	0.53	0.23	0.13	0.48	1.47
Lentil ..	N ..	1.70	4.10	0.23	0.39	0.85	4.07
	D ..	0.60	2.05	0.20	0.12	0.55	1.06
Capsicum ..	N ..	4.89	3.03	0.30	0.58	0.60	4.23
	D ..	2.35	1.90	0.31	0.12	0.59	2.01
Coriander ..	N ..	1.20	2.50	0.50	0.66	0.50	2.33
	D ..	0.10	0.44	0.08	0.18	0.47	0.88
Barley ..	N ..	1.25	0.40	0.16	0.42	2.02	..
	D ..	0.63	0.17	0.02	0.04	0.22	—

Water Supply

Tap water at the laboratory and the glass house was either from the town supply which was Gomti river water processed at the water works or tubewell water. The water from the town supply and the tubewell was purified by the following methods :

(i) Distillation :
  (a) in all copper stills.
  (b) in Kilburn 'Manesty' stills with a stainless steel condensor or in which the baffles and top cover were made of stainless steel.
  (c) Pyrex (or Corning) glass stills of the type in use at the Research Station, Long Ashton and described by Hewitt (1952).

(ii) Deionising by passing through ion exchange resins.

The ion exchange assembly comprised two Mark IV F and one Mark V F 'Permutit' deionising units, each containing 'Zeoarb HI'and 'Deacedite' resins. The units were so set that water passing from 'Deacedite' column of the first unit passed to the Zeocarb HI column of the scond and that from the Deacedite column of the second unit to the Zeocarb HI column of the third. In the later experiments the mark IV F units were replaced by Mark 8 units and the Mark V F by a mixed bed unit—Mark VI, manufactured in India by Ion Exchange India (Ltd.) under licence from 'Permutit' London.

Because of the high salt content in the water supply, (i) the deionising units needed regeneration after passing 500 to 800 L of water and (ii) before feeding in glass distillation stills water had to be deionised by passing through a single set of Deacedite and Zeocarb resins.

Table 6—Mineral nutrient element composition of water from the town water supply as purified by different methods.

Water sources	Mineral nutrient element									
	Ca	K	Mg	P	S	Fe	Mn	Cu	Zn	Mo
					mg/L					
River water (Gomti) ..	60.0	1.5	2.0	0.05	3.0	0.25	0.15	0.002	0.005	0.002
Tube well water	30.0	1.5	2.0	0.15	3.5	0.85	0.25	0.01	0.09	0.001
Water demineralised on ion exchange resins	0.10	0.19	0.01	4.3	0.01	<0.001	<0.001	<0.001	<0.001	<0.00001
Water distilled in copper stills	1.73	0.38	0.02	0.50	0.02	0.002	<0.001	0.54	0.005	0.00002
Water distilled in Manesty stills	<0.01	<0.01	<0.01	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	0.00002
Water distilled in all pyrex glass stills after demineralising by ion exchange re-resins ..	<0.01	<0.01	<0.01	<0.02	<0.01	<0.001	<0.001	<0.001	<0.001	<0.00001

On the basis of the analysis of the water supply from the different sources (Table 6) and culture trials, the type of water found suitable for obtaining particular nutrient element deficiencies was as follows :

(i) for obtaining deficiency of macronutrient elements—water from copper still, manesty still or deionised water.
(ii) for obtaining iron, maganese and copper deficiency effects—deionised water.
(iii) for obtaining copper, zinc, and molybdenum deficiencies—glass distilled water.
(iv) for obtaining boron deficiency-water from 'Manesty' stills containing lid and baffles made of stainless steel.

Except for copper and molybdenum deficiency work, water distilled in Manesty stills was almost as good as the deionised water. In case of copper deficiency the deionised water was as good as the glass distilled water but it was not so for zinc defficiency. Glass distilled water was found to be the best for work on micronutrients other than boron.

Culture Vessels

For producing deficiencies of macronutrients, iron, manganese, boron, copper and zinc, high density polythene containers (to which no colouring matter was added) were found satisfactory. Clay flower plots with a central drainage hole were found equally

suitable for macronutrients, iron and boron deficiency work provided they had been applied three coats of 'bitolin' or had been lined with alkathene. The bitumen paint had to be applied on alternate days to allow the coat applied earlier to dry and after paintings the pots had to be left in open to allow its volatile constituents to volatalise. For quantitative and refined work on copper and zinc and for all molybdenum deficiency work the low form 5 to 10 L beakers made of 'pyrex' or 'corning' glass and provided with a central drainage hole were found essential.

In the culture vessels, sand was retained by means of a pad of glass-wool kept beneath the rim of an inverted watch glass placed above the drainage hole. The watch glass and the glass-wool used were purified by boiling with 1 : 1 hot hydrochloric acid. Where glass vessels were used, they were first washed in hot water with a detergent and then immersed in 17% w/v hydrochloric acid, contained in large size jars for four hours. After taking out from the acid, the pots were rinsed with deionised water. The pyrex-glass pots were supported on wooden stands provided with deep circular holes in the centre. The stands were painted with bitumen.

The size of the pots used depended on the duration for which plants had to be maintained in culture. For most experiments, carried out to find out the macro- and micronnutrient requiremens of crop plants, 10 L pots were found satisfactory. These pots could take approximately 10 kg sand. Larger containers were required for long term experiments on sugarcane and plantation crops.

Nutrient Solution

Luxuriant growth of a wide variety of plants was obtained in sand culture using the nutrient solution described earlier. But this (nitrate type) solution was not found suitable for raising rice plants. Rice plants had to be supplied a mjor part of their nitrogen requirement in the ammonical form. In the culture solution for rice, therefore, calcium was supplied as chloride, potassium as sulphate and nitrogen as ammonium nitrate. If it was desired to supply more than 12 m. eq./L nitrogen, the additional nitrogen was supplied as sodium nitrate. In view of its higher availability in the chelated form, the source of iron supply was changed from ferric-citrate to ferric-EDTA prepared as described by Jacobson (1951). At the low and normal concentration of iron, Fe-EDTA was found a very suitable source of iron but at higher concentrations (>11.2 ppm) most plants grew better with ferric citrate than with Fe-EDTA.

Nutrient solutions were applied daily except on weak ends when pots were flushed with purified water. The solutions were gently applied to the surface of the sand near the periphery of the pots. The amount of the nutrients applied daily varied according to the season and the requirement of the plants. 0.5 to 1L of the nutrient solution was generally found sufficient to saturate the sand. When the requirement was high, plants were watered in the evening in addition to the nutrients applied in the morning.

For obtaining micronutrient deficiencies the elements in question were not only omitted from the nutrient medium but also the macronutrient salts and the source of iron supply—ferric citrate or Fe-EDTA—were appropriately purified (Hewitt, 1952). A. R. grade salts were used for the preparation of the macronutrient stock solutions. Calcium nitrate was prepared from A. R. grade calcium carbonte and A. R. grade nitric acid. The procedure for purifying the different macronutrients for obtaining particular micronutrient deficiencies is given in table 7.

Table 7—Methods for purification of macronutrients against micronutrients.

Micronutrient element removed	Methods used for removal*	Number of time purification procedure repeated
Iron and Manganese ..	Phosphate adsorption technique	Two
Copper and zinc ..	(*a*) Phosphate adsorption technique followed by ..	One
	(*b*) extraction at pH 6 with 0.05% dithizone solution in carbon-tetrachloride. Traces of carbon tetrachloride removed by volatalization.	Till traces of pink or blue green colour appeared in CCl_4 layer when dilute dithizone added.
Molybdenum ..	Co-precipitation with copper sulphate	Two
Boron	Recrystallisation	Two

*For the details of the methods see HEWITT (1952).

RAISING EXPERIMENTAL PLANTS

All plants were raised from seeds sown directly in the sand. By means of a clean glass rod (dia. 3 to 8 mm) holes were made in sand upto a depth of 1 to 4 cm depending upon the type of the seed sown. Clean seeds were put in these holes and the holes were loosely covered with sand with the help of a glass rod. As many as 20 seeds were sown in a pot. After the seedlings had emerged they were thinned to a uniform number in each pot. Subsequent thinnings were arranged to facilitate sampling.

With the help of the technique described above, large scale pot sand culture experiments could be undertaken for studying the macro- and micronutrient requirement of a wide variety of plants. At a time, more than a thousand pots receiving differential supply of macro- and micronutrient elements could be maintained under glass house conditions. The technique was found suitable for inducing severe symptoms of micronutrient deficiencies that can be of diagnostic value in identifying field disorders in several crop plants (AGARWALA & SHARMA, 1970; AGARWALA, unpublished), and for screening many of the newly released high yielding varieties for susceptibility to stress for particular micronutrients (AGARWALA & SHARMA, 1974; AGARWALA, SHARMA, SHARMA & NAUTIYAL, 1971; AGARWALA, unpublished). With suitable adaptations, the technique was also found suitable for investigating ion uptake problems using radioactive tracers. A continuous and a rigorous check on the methods described was, however, absolutely essential to overcome the serious problems of contamination from different sources.

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