

was also more satisfactory for *N*-nitrosodimethylamine than the heterocyclic nitrosamine, for which there were deviations in 18 out of 32 determinations.

For the types of materials listed in this report, it is concluded that the most satisfactory mass spectrometric procedure is that of high-resolution parent ion monitoring with peak matching. Chemiluminescence, although not responding exclusively to the nitrosamines studied, gave results in agreement with these mass spectrometric observations in all cases.

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An Insoluble Copper Phosphate in Copper Superphosphate

Robert J. Gilkes

When soluble copper salts are granulated with freshly made ordinary superphosphate, a compound $\text{NaCu}_6\text{H}_3(\text{PO}_4)_5\text{Cl}\cdot 6\text{H}_2\text{O}$ resembling the mineral sampleite is formed which is poorly soluble in water. The availability of fertilizer copper to plants growing in acid soils is probably not affected unless the compound is present as large inclusions. X-ray diffraction electron microprobe and infrared data for the compound are presented.

It is a common manufacturing practice to add micronutrients to a superphosphate carrier during granulation in order to promote uniform application of a few kilograms of micronutrients per hectare in the field (Silverberg et al., 1972). In many instances chemical reactions between the micronutrient salt and superphosphate occur, particularly when micronutrient additions are made during the granulation process when freshly made superphosphate contains considerable free acid. In some cases reactions may promote an increase in solubility of the micronutrient as in the case of zinc added as zinc oxide to concentrated superphosphate (Mortvedt and Giordano, 1969). Little is known of reactions between copper salts and super-

Table I. Solubility of Copper in Copper Ordinary Superphosphate (OSP)

	Total Cu, %	Water soluble Cu, %	Insoluble Cu, %
One 14 h water extraction ^a			
Crushed high copper OSP granules	1.2	0.6	0.6
Crushed blue-green scrapings	2.4	0.9	1.5
Crushed scraped high copper granules	1.0	0.5	0.5
Repeated water extractions ^a			
Crushed, average copper OSP granules	0.7	0.5	0.2

^a 0.1 g in 20 mL.

phosphate, although several possible reaction products have been proposed which are insoluble in water and may

Department of Soil Science and Plant Nutrition, The University of Western Australia, Nedlands, Western Australia 6009.

Table II. X-Ray Diffraction Patterns of Copper Phosphates

Fertilizer compound NaCu ₆ H ₃ (PO ₄) ₅ ·6H ₂ O, <i>d</i> , Å	JCPDS 1/6054 Cu ₃ (PO ₄) ₂ · 3H ₂ O, <i>d</i> , Å		JCPDS 12-520 CuHPO ₄ · H ₂ O, <i>d</i> , Å		JCPDS 11-349 sampleite, <i>d</i> , Å		<i>hkl</i> ^a		Synthetic copper phosphate ^b Cu _{5.74} H _{3.54} (PO ₄) ₅ ·4.2H ₂ O, <i>d</i> , Å	
	I	I	I	I	I	I			I	I
9.6	10	9.9	10	9.62	10	9.6	10	100, 040	9.5	10
6.83	3	6.9	3	6.80	5	6.85	7	140	6.75	2
				4.97	5					
4.72	1	4.80	1			4.73	4	002, 220	4.75	1
4.28	4	4.32	2			4.30	8	081, 211	4.28	3
		3.90	1	3.96	1	3.89	7	142, 260	3.93	2
3.36	1	3.34	1	3.36	1				3.32	<1
						3.23	5	0120, 310	3.21	4
3.03	10	3.02	9	3.06	8	3.04	10	291, 311	3.05	7
2.88	2					2.89	5	360, 272	2.91	2
						2.80	5	370, 282	2.83	3
2.66	2	2.65	2	2.66	5	2.69	5	380, 2120	2.65	2
2.57	1	2.54	1			2.57	5	342, 1122	2.57	1
2.48	1	2.49	1						2.48	1
2.42	3	2.41	1	2.41	1	2.40	5		2.42	1
2.35	1	2.34	1	2.34	1				2.34	1
2.16	2	2.15	1	2.14	3				2.16	1
		1.93	1						1.93	1
1.90	2			1.91	2	1.91	5		1.90	1
						1.79	7		1.77	1
1.702	5	1.71	2	1.71	5	1.71	8		1.70	1
		1.66	1	1.65	1					
1.613	2	1.61	1			1.61	5		1.62	1
1.510	2	1.50	1	1.50	1				1.52 } 1.50 }	2
						1.45	7			
1.438	1	1.43	1	1.43	1	1.44	7		1.43	1
1.369	2	1.37	1	1.37	1	1.37	7			

^a Orthorhombic indices (Hurlbut, 1942): $a = 9.70$ Å, $b = 38.70$ Å, $c = 9.45$ Å. ^b Omitting many weak lines.

be poorly available to plants (Lehr, 1972).

Laboratory examination of samples of Australian commercial copper-bearing, ordinary superphosphates (OSP) which had been manufactured by granulating CuSO₄·5H₂O with freshly made, acidic OSP have shown that a poorly water soluble, blue-green reaction product forms. This material is present both as a disseminated pigment throughout granules, including bright blue-green stained zones and as small (<0.05 mm), discrete inclusions.

The solubility of copper in copper superphosphate and in blue-green stained areas removed by scraping with a scalpel was assessed by a single 14-h water extraction and by repeated water extractions until all water soluble matrix components had dissolved (Table I). Approximately half the added copper was poorly soluble and on this basis might not be expected to be readily available to plants. The copper in the blue-green stained zones and, in particular, the inclusions were seen to be less soluble than the finely disseminated pigment. Similar inclusions have been recovered from fertilizers after long periods in the soil, confirming that the formation of insoluble copper compounds does limit the value of copper from copper OSP granules (Fiskell et al., 1967; Gilkes, 1975).

This investigation was undertaken to separate and identify the copper compound in Australian copper OSP so that its chemical and structural properties could be determined. Accurate identification was necessary before it could be synthesized for use in laboratory and glasshouse experiments to determine its immediate and longer term availability to plants.

IDENTIFICATION OF COPPER PHOSPHATE

Microscopic examination showed that the inclusions were in the form of microcrystalline aggregates that could not be identified optically. An electron microprobe an-

alyzer was used to map out the distribution of elements in polished sections of impregnated specimens of a commercial OSP containing approximately 0.6% copper and zinc. The specimens included examples of both fresh granules and granules recovered from soils. The latter were generally porous, friable residues consisting mainly of anhydrite, most phosphate and zinc having migrated from the granule shortly after its first contact with moist soil (Gilkes, 1977). The qualitative results of these analyses are shown in Figure 1 for a granule of fresh copper-zinc OSP. The matrix consists of a fine-grained mixture of calcium, phosphorus, and sulfur compounds, i.e., mainly anhydrite and monocalcium phosphate. Zones high in calcium and phosphorus with some fluorine but deficient in sulfur were frequently observed which are grains of apatite from rock phosphate that did not dissolve during manufacture of OSP. High zinc zones were rarely encountered, so most zinc is disseminated throughout the granules whereas much copper is present as inclusions in association with phosphorus, sodium, and chlorine. This association of elements is clearly seen in Figure 1.

X-ray diffraction (XRD) patterns were made with a Debye-Scherrer camera of inclusions separated under the microscope by hand-picking blue-green grains from the residue of repeated washings of crushed granules. They showed that the compound has a diffraction pattern resembling those of the mineral sampleite NaCaCu₅(PO₄)₄Cl·5H₂O and the synthetic compounds CuHPO₄·H₂O and Cu₃(PO₄)₂·3H₂O (Table II) (Guillemin, 1956; JCPDS, 1975; Hurlbut, 1942). Differences between the patterns may be due to a combination of variable crystallinity, preferred orientation, and the presence of unsuspected impurities in some standard samples. If the standard patterns are accepted as being completely reliable, the pattern of the fertilizer compound most resembles that of

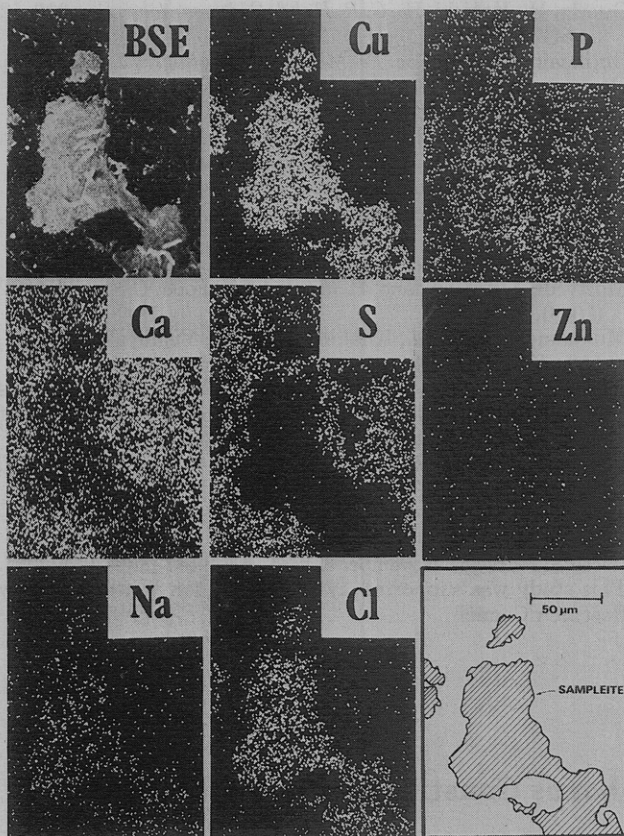


Figure 1. Electron microprobe analyzer x-ray images of a sampleite inclusion in copper-zinc OSP showing the association of copper, phosphorus, sodium, and chlorine in the inclusion (BSE back-scattered electrons).

$\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ since the strong high-spacing lines at 4.97 ($\text{CuHPO}_4 \cdot \text{H}_2\text{O}$) and 3.89, 3.23, 2.89 Å (Sampleite) are missing. The similar patterns exhibited by these chemically distinct compounds may indicate that they belong to the same basic structural type which is probably that of the mineral lavendulan $\text{Na}(\text{Ca,Cu})_6(\text{AsO}_4)_4\text{Cl} \cdot 4\text{H}_2\text{O}$ (Guillemin, 1956). There is no similarity between the diffraction patterns obtained for the fertilizer compound and the published patterns of the several copper phosphates that have been predicted as likely reaction products in the literature (Lehr, 1972). X-ray diffraction patterns of handpicked blue-green grains from unwashed fertilizer showed the same pattern accompanied by strong lines of anhydrite and monocalcium phosphate hydrate, indicating that the sampleite-like material did not form during washing.

Six milligrams of almost pure inclusions were separated for chemical analysis by exhaustive washing and hand picking under a binocular microscope. Very accurate analyses for major elements and water content could not be undertaken on such a small sample because of potentially large weighing errors. However, analysis of separate 3-mg subsamples by different analysts gave the same molar ratio Cu/P of 1.20. Sodium, chlorine, and loss on ignition were also determined semiquantitatively leading to an approximate structural formula $\text{NaCu}_6\text{H}_3(\text{PO}_4)_5\text{Cl} \cdot 6\text{H}_2\text{O}$.

The minor amounts (about 0.2%) of calcium and sulfate detected by chemical analysis were allocated to the anhydrite that was seen to be adhering to some grains.

The infrared absorption (IR) pattern for 2 mg of the compound mixed through a potassium bromide disk resembles those published for $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$ shown in

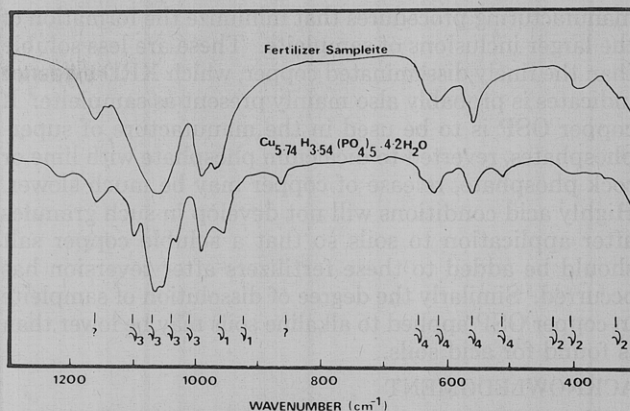


Figure 2. Infrared absorption patterns for fertilizer sampleite and synthetic $\text{Cu}_{5.74}\text{H}_{3.54}(\text{PO}_4)_5 \cdot 4.2\text{H}_2\text{O}$. Assignments for peaks are those given by Miller and Wilkins (1952) for $\text{Cu}_3(\text{PO}_4)_2 \cdot 3\text{H}_2\text{O}$.

Figure 2 (Miller and Wilkins, 1952; Vratny et al., 1961). There appear to be no published IR patterns for sampleite and $\text{CuHPO}_4 \cdot \text{H}_2\text{O}$ and pure samples were not available for comparison purposes. A specimen of $\text{Cu}_{5.74}\text{H}_{3.54}(\text{PO}_4)_5 \cdot 4\text{H}_2\text{O}$ was synthesized from copper hydroxide gel and concentrated phosphoric acid at 60 °C (Guerin and Kozicki, 1952a,b). This gave XRD and IR patterns similar to the fertilizer compound, although in both cases more detail was present and sharper peaks occurred (Figure 1). Sensitive x-ray diffractometry revealed many weak reflections not found for either sampleite or the fertilizer compound (Table II).

DISCUSSION

The above evidence shows that a water-insoluble acid copper phosphate resembling sampleite forms when copper compounds are added to freshly manufactured OSP. This is the first identification of this compound in superphosphate, although Russian workers have speculated that $\text{CuHPO}_4 \cdot n\text{H}_2\text{O}$ may form during the manufacture of mixed copper and ammonium phosphate fertilizers (Mukhammedzharov et al., 1970). The chlorine and sodium in fertilizer sampleite presumably originates in the rock phosphates used as raw materials (these contain up to 1000 ppm NaCl) and in water used in processing (up to 1000 ppm NaCl). Sampleite in copper OSP contributes less than 1000 ppm NaCl on a whole fertilizer basis and would presumably contain little NaCl when raw materials low in NaCl are utilized.

The behavior of fertilizer sampleite in the soil cannot be simply predicted from the solubility of the compound in water. Chemical analysis of copper OSP granules recovered from acid soils indicates that most copper is released from granules within a few weeks, although sampleite inclusions are often found in anhydrite relics of granules recovered from dry, sandy soils up to 2 years after application (Gilkes, 1975). The acid phosphate solution that develops in superphosphate granules in soils by rapid hygroscopic uptake of soil water may allow a much higher proportion of copper to dissolve than is predicted by simple water extraction. The ability of organic matter in soils to form strong complexes with copper may also promote more rapid dissolution (Hodgson, 1963).

Since the crystallization of sampleite in copper OSP does not prevent the release of most fertilizer copper to the soil, there is no necessity to suggest manufacturing procedures that will preserve the high solubility of copper sulfate pentahydrate, the commonest copper compound added to OSP. Some improvement in copper release from granules of copper OSP could probably be achieved by adopting

manufacturing procedures that minimize the formation of the larger inclusions of sampleite. These are less soluble than the finely disseminated copper, which XRD evidence indicates is probably also mainly present as sampleite. If copper OSP is to be used in the manufacture of superphosphates, reverted to dicalcium phosphate with lime or rock phosphate, release of copper may be much slower. Highly acid conditions will not develop in such granules after application to soils so that a soluble copper salt should be added to these fertilizers after reversion has occurred. Similarly the degree of dissolution of sampleite in copper OSP applied to alkaline soils may be lower than is found for acid soils.

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Effect of Phosphorus Fertilization on Phosphorus Constituents in Soybeans

Amin C. Kapoor¹ and Yogindera P. Gupta

Two varieties of soybean (Bragg and Punjab-1) grown in pot culture with three levels of phosphorus were studied for their chemical composition and different phosphorus compounds in the seed and its protein fractions. Crude protein, true protein, phosphorus, magnesium, and iron contents of the seed increased under the influence of phosphorus but the amount of nitrogen free extract and calcium decreased slightly. Phytic acid P constituted the major form of P in the seed followed by nucleic acid P and phospholipid P. Inorganic P formed a very small fraction of total P. The compounds containing the maximum amount of total P, phytic acid P, nucleic acid P, and inorganic P were mainly associated with the glycinin fraction of soy proteins. The concentrations of phosphorus compounds in the seed and its protein fractions were enhanced under the influence of soil phosphorus. The association of nucleic acid P with the major component of soy protein suggests their close interrelationship in metabolic activity and some soy proteins may be nucleoproteins.

Grain legumes are known to respond favorably to soil phosphorus. Phosphorus has been reported to influence the protein and phosphorus composition of soybean seed (Bhangoo and Albritton, 1972; Hanway and Weber, 1971). Soybean is a rich source of phosphorus-containing phytin, a calcium and magnesium salt of phytic acid as the main reserve phosphorus compound (Smith and Rackis, 1957; Sobolev, 1962) which has several physiological roles. Phytin is known for its chelating property with mineral elements in reducing their availability (Oberleas, 1973; Rackis, 1974; Lolas and Markakis, 1975). The other phosphorylated compounds are invariably associated in various vital processes in the development of the seed. Nucleic acids are known to play an important role in the

formation of seed proteins. It is reported that soybeans contain 1.3% ribonucleic acid suggesting that one or more of the proteins of soybean may be nucleoproteins (DiCarlo et al., 1955). In view of the importance of phosphorylated compounds in the grain legumes, the present investigation was undertaken to study the different phosphorus compounds in the soybean seed and their association with different protein fractions separated by solubility differences. The effect of soil phosphorus on the nutrient composition of seed was also studied.

MATERIALS AND METHODS

Two varieties of soybean (Bragg and Punjab-1) were raised in pots containing 12 kg of thoroughly mixed soil with a dose of 20, 40, 6, 4, 0.8, 20, 0.2, and 120 kg/ha of nitrogen, potassium, zinc, copper, boron, manganese, molybdenum, and sulfur, respectively, in five replicates in a completely randomized block design. Phosphorus was applied at levels of 0, 56, and 112 kg of P₂O₅/ha. Soil was sandy loam having pH 8.0, 0.23% organic matter, 5.6 kg/ha available P, and 134.4 kg/ha available K. Four plants were

Division of Agricultural Biochemistry, Indian Agricultural Research Institute, New Delhi-110012, India.

¹Present address: Central Grain Analysis Laboratory, Department of Food, Ministry of Agriculture and Irrigation, Krishi Bhavan, New Delhi-110001, India.