ABSORPTION AND TRANSLOCATION OF Zn^{65} IN PINTO BEAN SEEDLINGS GROWN IN NUTRIENT SOLUTION

By

ASSAD M. OKKEH

A THESIS

Submitted to the

AMERICAN UNIVERSITY OF BEIRUT

In partial fulfilment of
the requirements for the
degree of

MASTER OF SCIENCE
IN
BIOLOGY

May 1968

ABSORPTION AND TRANSLOCATION OF Zn^{65} IN PINTO BEAN SEEDLINGS GROWN IN NUTRIENT SOLUTION

By

ASSAD M. OKKEH

Approved:

John I. Mirhij

John I. Mirhij: Professor of Biology and Chairman of the examining committee.

Najib Abu-Haydar

Najib I. Abu-Haydar: Associate Professor of Internal Medicine. Faculty of Medical Sciences.

Usama al-Khalidi

Usama A. al-Khalidi: Associate Professor of Biochemistry, Faculty of Medical Sciences.

Date Thesis is presented: May 27, 1968.

ABSORPTION AND TRANSLOCATION OF Zn^{65} IN PINTO BEAN SEEDLINGS GROWN IN NUTRIENT SOLUTION

OKKEH

AN ABSTRACT OF THE THESIS OF

Assad M. OKKEH for the M.S. degree in Biology

Title: Absorption and translocation of Zn^{65} in Pinto bean seedlings grown in nutrient solution.

In this investigation some factors influencing the absorption and translocation of Zinc-65 in Pinto bean seedlings grown in nutrient solution were studied.

It was found that the presence of cations other than Zn^{++} or the lower pH value of the solution decreased zinc-65 absorption, but favored the translocation of zinc-65 to the shoot. Treatment of the roots with 0.01 M $CaCl_2$ up to 60 minutes showed no significant decrease in the zinc-65 content of the root. This treatment caused a marked decrease in the translocated zinc-65 to the shoot. There was no decrease in the rate of zinc-65 absorption during a period of 128 minutes.

An increase in the concentration of the stable zinc up to 50 ppm in the nutrient solution caused a corresponding decrease in zinc-65 absorption by the plants. An increase in the concentration of zinc-65 up to 14 mcu/liter, in distilled water and in complete nutrient solution, caused an increase in the absorption and translocation of the element.

Previous treatment of the Pinto bean plants with stable zinc for one week showed that the highest absorption of zinc-65 occurred when the plants were previously grown in a nutrient solution containing 2.5 ppm of stable zinc.

The maximum absorption of zinc-65 occurred when Pinto bean seedlings were grown in nutrient solutions having a pH range from 6.2 to 7.2. The highest zinc content in the root was found at pH 7.0. Maximum translocation to the stem and the leaves was at the acidic pH values. The soil samples collected at random from different areas of Lebanon were all alkaline having a pH level from 7.8 to 8.65. The highest zinc-65 absorption by Pinto bean seedlings was form the soil which had the least pH value. Translocation of zinc-65 to the shoot was highest at low pH values.

The highest accumulation of zinc-65 in the roots was found to occur in plants grown in complete darkness for 24 hours. Natural lighting conditions caused the highest translocation of zinc-65 to the leaves. Continuous illumination with an intensity of 3000 lumens did not cause the highest zinc-65 absorption, but favored the translocation of zinc-65 to the stem.

Primary leaves exposed to light accumulated in 48 hours 7 times as much zinc-65 as accumulated by the covered opposite leaves on the same plants.

Diurnal variation was found to occur in Pinto bean seedlings as to absorption and translocation of zinc-65. The highest root absorption occurred at 2 A.M. to 6 A.M., while the highest translocation to the stem took place from 6 A.M. to 2 P.M. Translocation to the leaves was best during the day hours.

Raising or lowering the temperature from 15° to 25°C. or from 25° to 15° caused no or little change in zinc-65 absorption by Pinto bean seedlings. Raising the temperature from 5° to 15°C. caused an increase in zinc-65 absorption. Lowering the temperature from 15° to 5°C. caused a decrease in zinc-65 absorption. The higher temperatures (25° and 15°C.) favored zinc-65 translocation to the shoot. The Q_{10} (from 5° to 15°C.) obtained was 1.5.

The highest absorption and accumulation of zinc-65 was found to occur in the roots and stems of four-week old plants. Younger leaves, primary and compound, accumulated more zinc-65 than older ones.

The autoradiograms showed that the root always contained a higher concentration of zinc-65 than the shoot. Along the stem, more zinc-65 accumulated in the nodes or at the bases of leaves.

More zinc-65 was absorbed by the lower surface of the leaf than the upper surface. More zinc-65 was absorbed when applied to the basal region of the upper surface of the leaf than the apex. But, more zinc-65 was translocated to the stem and the root when applied to the apical region of the upper surface than the basal region.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

The importance of zinc in plant and animal life is repeatedly emphasized by successive investigations. Since the essential nature of this trace element in living organisms was established during the first decade of this century, a great deal of research work was directed to throw more light on the metabolic role of this element.

The use of radioactive Zinc-65 as a tracer in various physiological and biochemical experiments provided a valuable contribution in elucidating some aspects of the absorption and utilization of zinc by living cells.

The literature contains an enormous number of papers on zinc in relation to plants. Most of these deal with the indispensability of the trace element to various species of plants, the symptoms of deficiency and toxicity, the requirement of some plants for the element, and in part with its metabolic role as manifested by its activating or inhibitory effects on some enzymes.

Only a few papers in the literature describe these aspects in bean plants, and still fewer are the papers that have dealt with zinc-65 in Pinto beans.

The influence of soil reaction on the availability and absorption of zinc, although known for a long time as shown by field and laboratory experiments, has a special importance in this part of the world due to the occurrence of alkaline soils. The optimum pH level described for the maximal zinc absorption in some plants is in fact a wide range, i.e. from 5.5 to 7.5. This was not done for many plants. A more specific work is needed to narrow down this wide range for some economic plants, especially in regions known for their alkalinity or zinc unavailability. The use of culture solutions alone, in this regard, does not seem quite applicable to natural soil. This necessitates the performance of parallel experiments on natural local soil to make the interpretation of results and the conclusions obtained applicable and beneficial in daily life.

There is no mention in the literature of the role of some factors that may have some influence on the availability and absorption of zinc in Pinto bean plants such as light, temperature, diurnal variation, nonroot nutrition, and the like.

Since the topical application of nutrients to certain plants is increasingly used, the translocation and distribution of the absorbed zinc from both root and nonroot supplementary nutritions need more attention and experimentation.

The present investigation was initiated in an effort to study the influence of some factors that affect the absorption and translocation of Zn^{65}, in the form of $Zn^{65}Cl_2$, by Pinto bean seedlings grown in nutrient solution.

II. REVIEW OF LITERATURE

Historical

The essentiality of zinc for the normal growth of the Red Kidney beans, Phaseolus vulgaris L., was reported by Sommer (1928), who, in her report, confirmed the results obtained in similar experiments on plants of different families by Sommer and Lipmann (1926) and Sommer (1927).

But, the chronological order of studying zinc in various organisms is as follows: As early as 1869, Raulin showed that zinc was essential in the culture medium for the normal growth of Aspergillus niger van Tiegh. Soon thereafter, zinc was shown by Lechertier and Bellamy (1877) and Raulot and Breton (1877) to be present in the tissues of plants, animals and man. Since that time, zinc has been classified as a "trace element", sometimes called micronutrient or oligo-nutrient, and has been the subject of nutritional, physiological, toxicological and biochemical investigations.

Javillier (1908) showed that the addition of zinc to purified cultures in which Aspergillus niger van Tiegh., was growing, increased the dry weight of that fungus. He located zinc in forty five species of flowering plants and suggested that zinc was essential to plants. The same results were

obtained by Bertrand and Javillier (1911), and confirmed very carefully by Steinberg (1919). Voelcker (1913) found that zinc stimulated the growth of maize plants. A year later, Maze' (1914) showed that zinc was essential for the normal development of maize plants.

The requirement of higher plants for zinc was indicated first from the work of Maze' (1914) for maize Zea mays L., and later beyond all doubt by Sommer and Lipmann (1926) and Sommer (1927, 1928) for barley Hordeum vulgare L., sunflower, Helianthus annuus L., buckwheat, Fagopyrum esculentum Moench., broad bean, Vicia faba L., and Kidney bean, Phaseolus vulgaris L. Steinberg (1926) also proved that zinc was indispensable to the normal growth of fungi. Investigations with yeast by McHargue and Calfee(1931) showed that zinc sulfate, among other substances, in small quantities, increased the dry weight of yeast produced, and it was found that excessive quantities of those sulfates were toxic resulting in decreased growth or death of the cells. The work of Porges[1](1932), using Aspergillus niger van Tiegh., although overlooked by many authors who reviewed the earlier literature on zinc importance in plants, showed that zinc played an important part in the nutrition of the organism.

---

1. Porges, N., 1932, Botanical Gazette 94: 197 - 205.

The addition of zinc sulfate in the concentration of 0.01% repressed spore formation, but favored the vegetative phase of growth and caused greater utilization of the available sugar, resulting in an increased yield of the dry mycelium and a greater production of citric acid.

Meanwhile, evidence for the need of zinc in the field started to accumulate. In experiments on Florida peat soils, Allison, Bryan and Hunter (1927) found that zinc was one of the elements to which crops in that region responded. Fruit trees, including citrus, had been subject to a chronic disturbance characterized by poor growth and a dwarfing and rosetting of the leaves. Tung and pecan trees were also affected. It was not until the early thirties that an insufficiency of availability of zinc was found to be the cause of the trouble. A series of experiments by Chandler and his associates (1931, 1932, 1933, and 1934) on fruit trees, established the need for zinc. Mowry and Camp (1935) with corn and other crops, obtained the same conclusions. Husz (1940), described for the first time in Europe the rosette disease of apples growing in Hungary. He was able to cure the deficiency by an application of zinc sulfate. It was demonstrated by Smith and Bayliss (1942) that zinc was necessary for Pinus radiata Don., Plants grown in nutrient solution purified with respect to zinc, showed deficiency symptons in three to four months. Camp (1945) reported the occurrence of zinc deficiency on citrus plants in Egypt and Palestine.

Zinc in animals

Zinc was found consistently in animals, both vertebrate and invertebrate. Mendel and Bradley (1905) showed that zinc was a constituent of hemosycotypin, the respiratory pigment of the snail Sycotypus. Hiltner and Wichmann (1919) demonstrated the presence of zinc in oysters. Bodansky (1920) showed that zinc was present in marine organisms.

A zinc deficiency in the rat was first observed by Todd et al.(1934). Stirn et al.(1935) established the indispensability of zinc in the nutrition of the rat, and the results were confirmed three years later by Hove et al.(1938). The effects of dietary zinc deficiency were studied later in the rat by Day and McCollum (1940) and in the mouse by Day (1942) and Day and Skidmore (1947).

Zinc deficiency in animals results in a greatly retarded growth rate, emaciation, loss of hair from head and shoulders as reported by Elvehjem and Hart (1934), parakeratosis by Thomas (1965), a slow absorption of both carbohydrate and protein from the gastrointestinal tract which indicates a decrease in the efficiency of converting food into body tissues by Gilbert (1957). According to White et al.(1959), zinc deficiency in the rat is manifested by retarded growth, alopecia and lesions in the skin, esophagus and cornea. They also reported that hogs develop

parakeratosis with anorexia, nausea and vomiting and that this disease is readily cured with inclusion of 0.02% zinc carbonate in the diet. A resistant deficiency in witamin A was shown by Stevenson and Earle (1956) to exist in porcine parakeratosis. In addition to the above mentioned symptoms, Macapinlac et al.(1966) observed that zinc deficiency in the rat caused lymphocytopenia and increased hematocrit values. Concentrations of zinc in the bone and testes were markedly decreased. The testes showed atrophy and degeneration of the germinal epithelium, but the ovaries exhibited no changes. The weight of the spleen was markedly decreased.

The importance of zinc in Protozoa was studied by Price and Quigley (1966). They used Euglena gracilis Klebs, cells cultured in media containing Zn^{65} and limiting amounts of Zn. The results obtained indicated that the growth rate of the organisms, measured as protein, was a linear function of the internal zinc concentration of the cells.

The presence of zinc in the tissues of the horse was reported by Bertrand and Vladesco (1921, 1922). O'Dell and co-workers (1932) showed that zinc is required for growth and various other functions in birds.

Zinc in man

Zinc in human tissues was reported by Ghigliotto (1919) and Giaya (1920) long before its essentiality was proved. Later, marked abnormalities have been described in a widely prevalent liver disorder, postalcoholic cirrhosis by vallee et al.(1956, 1957). The concentration of zinc in the serum of these patients is markedly decreased. In cases with "severe" cirrhosis the serum zinc concentration is 66.6 ± ug/ 100 ml., as compared to the normal 121.0 ± 19. The zinc content of liver of individuals dying with postalcoholic cirrhosis in also decreased as shown by Lundegardh and Bergstrand (1940) and Vallee et al. (1957). In cirrhotic patients 1 gm of wet liver tissue contains 29 ± 7 ug of zinc, as opposed to 74 ± 23 ug/gm in individuals dying from other causes.

Zinc has long been known to be present in the retina of many species in very high concentrations as shown by Bowness et al. (1952). Underwood (1962) reported that the highest concentrations of zinc known to exist normally in living matter occur in the choroid of the eye of a wide range of animal species. Vitamin A metabolism, as manifested by abnormal dark adaptation, was found by Patek and Haig (1939) to be altered in human cirrhosis. Witamin A_1 alcohol dehydrogenase and liver alcohol dehydrogenase, which appear to be

the same enzyme according to Bliss (1951), contain 0.18% of zinc.

Leukocytes of the human blood were reported by White et al. (1959) to contain a high concentration of zinc which is about 25 times that in the erythrocytes, this is well justified since, unlike the erythrocytes, leukocytes possess well organized systems of both respiratory and glycolytic enzymes.

Prasad (1966) found that zinc deficient patients exhibited severe growth retardation and sexual hypofunction. The endocrine manifestations associated with human zinc deficiency, as reported by Sandstead (1966), were compatible with "Hypopituitarism" and included: a) growth failure and retarded osseous development, b) hypogonadism, c) apparent depression of endogeneous ACTH production, d) and increased sensitivity of insulin. These clinical features were improved and puberty induced following zinc treatment. Halsted (1966) reported that zinc deficient patients, in Egypt, showed dwarfism, hypogonadism and iron deficiency anemia.

According to Callender and Gentskow (1937) and Ormrod (1943) cases of zinc poisoning of domestic animals and persons are very rare. It was found by Hegsted et al. (1945) that the levels at which zinc compounds must be fed to produce acute toxic effects were high for all the animals studied. Gilbert (1957) reported that consumption of excessive zinc may be harmful in two ways: First, since zinc competes with copper, too much zinc or too little copper decreased the production of ascorbic acid oxidase and tyrosinase. Secondly, zinc interferes directly with the manufacture of cytochrome oxidase,

which is important in utilizing copper for hemoglobin production.

As reported by Van Reen (1966), zinc is present in the various tissues of man and other vertebrates in concentrations varying from 10 - 200 ug/gm. Studies of Widdowson et al.(1951) indicated that a 70 Kg man contains 1.36 to 2.32 gm of zinc. Van Reen (1966) also found that toxicity symptoms can be caused by the ingestion of 225 - 450 mg Zn^{++} . The usual symptoms of toxicity are fever, nausea, vomiting, stomach crumps, and diarrhea in 3 - 12 hours following ingestion.

Metabolic role of zinc

Zinc has been reported to play significant roles in fundamental life processes as shown by various experiments on plants, animals and man.

The relationship between zinc and auxin contents of many plants was shown by many investigators. According to Skoog (1940), Hoagland (1944), Tsui (1948) and Nason *et el.* (1951), a zinc deficient plant shows consistently a low auxin content, which some authors relate to tryptophane deficiency. Tryptophane is believed to be required for auxin synthesis. Skoog (1940) showed that tomato *Lycopersicon esculentum* Mill., plants deficient in zinc were deficient in auxin.

In animals and man, the report published by Scott (1934), and later by Scott and Fisher (1938) led to speculation that there might be some functional relationship between zinc and the elaboration of insulin by the pancreas and even in the function of insulin *in vivo*. More recently, according to Orten (1966), histochemical studies revealed the occurrence of variable amounts of zinc in both the alpha and beta cells of the islets of Langerhans. The amount of zinc in the beta cells varied directly with insulin secretion in response to physiological stimuli.

Orten (1966) concluded that, so far, there was only indirect evidence for the possible relation of zinc to the following hormones: insulin, glucacon, adrenocorticotrophic (ACTH), the growth hormone, gonadotrophin and perhaps testosterone. Orten believes that zinc may also be involved in the synthesis of RNA and hence in protein synthesis. Pries and Strain (1966) found that zinc played an important role in promoting wound healing in animals and human beings.

The metabolic role of zinc in various organisms can best be demonstrated by the occurrence and importance of zinc in many enzymes of plants and animals. The functional interaction of zinc with enzymes may be defined as falling into one of two categories: either metal-enzyme complexes or metalloenzymes. Although the metal-enzyme complexes, apparently involving zinc, are numerous as reviewed by Hoch and Vallee (1958) and others, they were studied extensively <u>in vitro</u> only. The activating effect of the zinc ion upon an enzyme <u>in vitro</u> defines neither the structural association with the enzyme nor a unique metabolic function of the metal. But, zinc metalloenzymes are defined to incorporate zinc into the protein matrix so firmly that the two can be thought of as an

"entity" and they can be isolated, due to their specificity and firmness of binding of zinc and protein matrix.

A brief review of enzymes containing zinc or activated by it is given in the following table. More additions to this list are likely to be made later.

Enzymes containing, or activated by, zinc

Enzyme	Authors and dates	Source	Comments
1. Alanylglycine dipeptidase.	Orten (1966)		
2. Alcohol dehydrogenase (YADH).	Negelein & Wulff(1937)	Yeast	First to be crystallized
	Vallee & Hoch (1955)		4 Zn atoms/enzyme.
	Vallee & Gilson (1948)		Mol. Wt. 150,000.
	Hoch & Vallee (1949)		
3. Alcohol dehydrogenase (LADH)	Bonnichsen & Wassen(1948)		First crystallized.
	Bonnichsen (1950)	Equine	Mol. Wt. 7300.
	Bliss (1949, 1951)	liver	2 Zn atoms/enzyme.
			less specific than YADH,
			oxidizes vit.
			A alcohol
	Holzer & Schneider(1955)		Oxidizes glycerol.
	Theorell & Bonnichsen(1951)		Reacts with 2 DPN at
			neutral pH.
	Von Wartburg et al.(1964)	Human liver	
4. Aldolase	Quinan-Watson (1951)	Higher plants	
	Rutter & Ling (1958)	Yeast.	
	Jaganathan et al.(1956)	Aspergillus	

Enzyme	Authors and dates	Source	Comments.
5. Alkaline phosphatase	Baily & Webb (1948)	Animal tissues	
	Mathies (1954,1958)	Mammalian kidney,	Purified enzyme contained 0.15% Zn.
	Dixon & Webb (1958)		Zn.has nothing to do with the activity.
	Plocke et al.(1962)	E. coli	
	Trubowitz et al.(1961)	Human leulocytes.	
6. Aminopeptidase.	Orten (1966)		
7. Arginase	Dixon & Webb (1958) Orten (1966)	Animal tissues.	
8. Argininase	Boyer et al. (1959)		
9. Arginine desimidase	Colowick & Kaplan (1955)	Bacteria, Yeast.	
10. ATPase	Boyer et al.(1959)	Erythrocytes.	
11. Carbonic anhydrase.	Brinkman et al.(1932)		First described as a protein catalyst.
	Keilin & Mann(1939,1940)	Bovins erythrocytes	Zn was established as a metal component contains 0.33% Zn.
	Day & Franklin (1946)	plants	Plant enzyme also contains Zn.

Enzyme	Authors and dates	Source	Comments.
	Dixon & Webb (1958) Lindskog & Malmstrom(1962)	Animal tissues.	
	Kickli & Edsall (1961)	Human erythrocytes.	
12. Carboxylase	Stumpf (1945)	Proteus vulgaris	
13. α Carboxylase	Bhatia et al.(1955)		
14. Carboxypeptidase A.	Arnon (1937) Vallee & Neurath (1954,1955,1964) Vallee (1955)	Bovine pancreas.	First crystallized 1 Zn atom/enzyme.
15. Carboxypeptidase B.	Folk et al.(1960) Wintersberger et al(1962)	Porcine pancreas Bovine pancreas.	
16. Carnosinase	Garkovi (1938)	Spleen kidney liver.	
17. Citrullinase	Knivett (1953) Slade (1953) Korzenovsky (1953) Oginsky & Gehrig (1953)	Bacteria	The Mg requirement can be replaced by Zn^{++}. Specific for L-citrulline.
18. Cyctochromes	Neilands (1957)	Ustilago sphaerogena.	Zn is necessary for synthesis.

Enzyme	Authors and dates	Source	Comments
19. Dehydropeptidase	Yudkin & Fruton(1947)	Animal.	
20. Dialkylfluoro phosphatase.	Boyer et al. (1959)		
21. Dipeptidase	Cohnheim (1901)		Discovered and called "erepsin", later found to be a mixture of enzymes.
	Linderstrom-Lang (1929, 1930)	Plants; Animals. micro-organisms.	Activated by small concentrations of Zn, While large concentrations of Zn inhibit the enzyme.
22. Enolase	Warburg & Christian (1941) Sumner & Somers(1943)	Animal tissues Yeast.	Crystallized as mercury salt. A Mg-protein complex, Zn can replace Mg.
23. Flavokinase	Davis & Gale (1953)	Baker's Yeast Intestinal mucosa.	
24. Formic dehydrogenase	Davidson (1951)	Peas	Inhibited by azide and cyanide.
25. Glucose 6-phosphate dehydrogenase.	Lamb et al. (1958) Dixon & Webb (1958) Steward (1963)	Yeast. Man Animals.	

Enzyme	Authors and dates	Source	Comments
26. Glutamic dehydrogenase	Vallee et al.(1955) Adelstein & Vallee (1956, 1958)	Beef liver	Average Zn content: 3.4 ± 1.0 atoms/enzyme
27. Glyceraldehyde 3-phosphate dehydrogenase.	Lamb et al.(1958) Steward (1963) Vallee et al.(1956)	Yeast Rabbit muscle. Yeast	
28. Glycerol dehydrogenase	Winder & O'hara (1961,1962,1963)	Mycobasterium smegmatis.	In Zn-deficient medium, enzyme activity fell to about a third after 3 days.
29. α-glycero phosphate dehydrogenase.	Lamb et al.(1958) Steward (1963)	Rabbit muscle.	
30. Glycylglycine peptidase	Steward (1963) Orten (1966)	Animals.	
31. Glycyl-L-Leucine dipeptidase.	Altenbern & Housewright(1953) Orten (1966)	Animal tissues	Uterine enzyme manifests maximal activity with Zn^{++} and phosphate.
32. Hexokinase	Medina & Nicholas(1957)	Neurospora crassa.	
33. Histidine deaminase.	Orten (1966)		

	Enzyme	Authors and dates	Source	Comments.
34.	Isocitric dehydrogenase	Kornberg & Pricer(1951) Langer & Engel(1958) Broyer et al. (1960)	Yeast	Inhibited by azide and cyanide. Contains Zn as a fuctional constituent Zn may serve as an organizer of reversible attachment between apoenzyme and coenzyme.
35.	B-Lactamase II	Kuwabara & Abrahams (1967)	Bacillus cereus	Dialysis resulted in complete loss of activity more than 80% of the activity was restored by the addition of Zn^{++}.
36.	D(-)Lactate cytochrome C reductase.	Orten (1966)		
37.	D-lactate dehydrogenase.	Price (1961) Dixon & Webb (1958) Vallee & Wacker(1956) Beisenherz et al.(1953)	Euglena gracilis Animal tissues. Rabbit muscle. Rabbit muscle.	
38.	Lecithinase	Orten (1966)		
39.	Leucylglycine dipeptidase	Orten (1966)		
40.	Malate dehydrogenase	Harrison (1963)	Porcine heart.	

Enzyme	Authors and dates	Source	Comments.
41. Metaphosphatase	Colowick & Kaplan (1955)	A.niger animal tissues.	
42. Nucleic acid hydrolyzing enzymes.	Brownhill et al.(1959)	Yeast.	
43. Neutral protease	Orten (1966)		
44. Oxaloacetate decarboxylase	Bidwell & Van Heynengen (1948)		
45. Phosphatase	Sadasivan (1950)	Penicillium Chrysogenum.	
46. Polyphenol oxidase	Shkol'nik & Abdurashitov(1959)	corn	
47. Pyruvic carboxylase	Foster & Denison (1940)	Rhizopus nigricans	Zinc is necessary for the synthesis of the enzyme, not a component.
48. Tripeptidase	Orten (1966)		
49. Tryptophane desmolase	Orten (1966)		

Enzyme	Authors and dates	Source	Comments
50. tryptophane systhetase	Nason et al.(1951)	Neurospora	
51. Uricase	Holmberg (1939)		Contains 0.13% Zn.
	Mahler, Hubscher & Baum (1955)	Kidney	Contains 0.05% Cu.
52. Zymohexase	Warburg & Christian (1943)	Yeast	
53. Adenylyl carbonate pyro-phosphorylase (H-enzyme)	Bachhawat, B.K.& Coon, M.J. (1957)	Heart	
54. Anserinase	Jones, N. R. (1955)	Fish muscle.	
55. Phospho-glyceraldehyde dehydrogenase	Boyer, P.D. & Segal, M.L. (1955) Caputto, R. & Dixon, M. (1945)	Widely distributed.	
56. Streptomyces protease A.	Tytell, A. A. et al. (1954)		

On the other hand, zinc exerts inhibitory effects on some other enzymes. The following were found to be inhibited by zinc ions:

1. Alkaline phosphatase.
2. Amino acid amidase.
3. Arginine desimidase.
4. Aspartase.
5. ATP-creatine transphosphorylase.
6. Creatine phosphokinase.
7. 5-dehydrogenase.
8. DNase.
9. DPNH cytochrome C reductase.
10. Fructose diphosphatase.
11. Glutamic dehydrogenase
12. Glycylglycine dipeptidase
13. Histidase.
14. Imidodipeptidase.
15. Leucine aminopeptidase.
16. Pantothenate-synthesizing enzyme.
17. Prolidase.
18. RNase.
19. D-serine(D-threonine)dehydrase.
20. Tryptophan synthetase.

Zinc deficiency in plants

According to Rogers et al. (1939), plant susceptibility differs considerably to a lack of available zinc. Some plants such as beans, soybeans, tomatoes and corn among herbaceous plants, show high sensitivity to zinc deficiency. Symptoms of chlorosis may begin to show within a week after the emergence of the seedlings from the zinc deficient soil. The full development of chlorophyll scarecely takes place in the older leaves before light-yellow or whitish streaks appear between the veins. The young unfolding leaves are almost entirely without chlorophyll.

Zinc deficiency symptoms in beans and soybean plants were described by Sprague (1964). Zinc deficient plants fail to develop to natural size, the interveinal areas of the leaves become yellow and chlorotic, the chlorosis being more severe on the lowest leaves. The chlorotic tissues may turn brown or grey and die prematurely. Few pods are produced. In cowpeas, small brown spots develop on the lower leaves. Interveinal areas become chlorotic and veins remain green. Tissues in the brown spots may die and edges of the leaves may become crinkled. Zinc content in deficient plants is low when compared with healthy plants. In soybeans, healthy plants contain 30 parts

per million of zinc, while deficient plants contain 20 parts per million or less. Alfalfa healthy plants contain 40 parts per million while deficient plants contain 15 parts per million of zinc or less. Viets et al. (1954) also found that deficiency symptoms are associated with about 20 parts per million of zinc or less in the mature leaves or the total top.

Histological changes in zinc-deficient leaves were described by Reed and his associates (1934, 1935, 1938, 1940 and 1942). Cell growth rather than cell multiplication is promoted in the palisade parenchyma, and in tomato the mesophyll appears to atrophy to some extent. There is also a lack of differentiation. Destruction of chloroplasts occurs and the greatest injury appears to occur in the cells most strongly illuminated. With zinc deficiency, some of the most striking changes in amino acid patterns occur. Possingham (1956) observed a tenfold increase in total amides and double the total free amino acid level in zinc-deficient tomato leaves. Steinberg et al. (1960) also found that zinc deficiency in tobacco caused a large increase in asparagine, glutamine and arginine, and decreases in α-aminobutyric acid and γ-alanine.

Zinc deficiency symptoms, according to Gilbert (1957), differ in herbaceous and woody plants, but they are remarkably

similar in plants within one group. Chlorosis is the most outstanding characteristic in herbaceous plants, while abnormalities of the twigs and leaves come in the first place in woody plants. In all cases growth is greatly retarded. Gilbert (1957) described zinc deficiency in woody plants and mentioned that clusters of small stiff leaves develop on twigs that have extremely short internodes. Frequently mottled chlorosis accompany these abnormalities. The disease may be known as "rosette" or "little leaf". Riceman (1948) found that zinc deficiency drastically decreased flowering and/or seed production. Viets (1966) noticed that zinc deficiency usually hinders reproduction more than vegetative growth.

The effect of zinc deficiency in bacteria was studied by Winder and O'hara (1961, 1962). They found that Mycobacterium smegmatis Alvares and Tavel, became elongated in zinc-deficient medium, and observed a decrease in RNA and DNA levels before growth was inhibited. In a more recent report, Winder and O'hara (1966) found that when growth was limited by zinc-deficiency in static cultures, the bacteria contained a mean of 11.3 ug of zinc/gm dry weight, which probably approximated the concentrations needed by the bacteria.

A deficiency of zinc in microorganisms was reported by Steward (1963) to result in an upset of metabolism. Thus, in experiments on Neurospora crassa Shear and Dodge, Nason et al. (1951) reported that alcohol dehydrogenase and tryptophan synthetase were reduced. In experiments, performed by Medina and Nicholas (1957), hexokinase, in the same organism, was also reduced in felts deficient in zinc. In Rhizopus nigricans Ehr., deficient in zinc, Vallee (1960) noticed a decrease in pyruvic carboxylase. Foster and Denison (1940) found that pyruvic carboxylase was absent in zinc-deficient Rhizopus nigricans Ehr.

Zinc toxicity in plants

Plants differ in their zinc tolerance. Although there is no general agreement on the concentration of zinc which exerts toxic effects in plants, excess of zinc was reported to induce symptoms of toxicity in various plants.

Zinc toxicity in soybean plants was first described by Earley (1943) as follows: "A red pigment forms at the base of the middle vein of the leaf and the leaves begin to curl under. Chlorosis of the tripartite leaves toward the upper part of the stem appears, the stem apex dies and the red pigment becomes more intense in the leaf veins, leaf petioles and the stem". Earley (1943) also found that even varieties of the same species may differ significantly in their sensitivity to excess of zinc. In soybeans, he found that one variety, Peking, tolerated a concentration of 0.1 ppm of zinc, but was damaged by a concentration of 0.2 ppm in the medium, and it was killed by a concentration of 0.4 ppm. While plants of the other variety, Hudson Manchu, tolerated zinc in a concentration of 0.8 ppm and were killed by a concentration of 1.6 ppm. Gilbert (1957) concluded that many plants will readily tolerate 0.1 ppm of zinc in water culture and probably 0.25 ppm in soil culture. But Brenchley (1914) regarded approximately 0.08 and 0.16 ppm of zinc as thresholds for toxicity

to barley and peas respectively, grown in water cultures.

In citrus plants, Guest and Chapman (1944), found that 2 - 4 ppm of zinc were toxic, but Haas (1932) reported that although slightly toxic to young citrus cuttings, 5 ppm of zinc benefited larger ones. In a later work by Haas (1949) the level of zinc was much reduced.

Optimum zinc requirement

The optimum concentration of zinc in water and soil cultures is still a subject of dispute. Kendall (1955) used 0.9 ppm of zinc in the nutrient solution for Phaseolus vulgaris L, variety Black Valentine. Wallace and coworkers (1963) used 0.12 ppm of zinc in nutrient solutions for Phaseolus vulgaris L, variety Tendergreen. Scharrer and Jung (1957) showed that the optimum requirement for zinc was 0.05 ppm of zinc in bean plants. Eaton (1941) supplied zinc to tomato and maize plants at 0.2 ppm in water cultures. Piper (1941, 1942) gave 0.2 ppm of zinc to cereals, peas and legumes in water cultures. Hewitt (1966) considered 0.02 to 0.2 ppm of zinc an adequate range for the majority of plants and he suggested 0.05 ppm of zinc for the firs trial with plants. This proposed concentration was previously used by Arnon and Hoagland (1940). Brusca and Haas (1959), utilizing variable zinc concentrations in sand culture by application of $ZnSO_4$ at levels more than 0.2 ppm, found that zinc prevented leaf mottling and stimulated the growth of lemon cuttings and avocado seedlings. The optimum zinc concentrations were around 5 ppm, but concentrations as high as 10 - 15 ppm of zinc still showed beneficial effects.

Zinc availability and absorption

Soils vary widely in their zinc content, with a range from a few to over 100 ppm, as reported by Holmes (1943). Swaine (1955) considers normal soils to have 10 to 300 ppm total zinc. But, it is obvious that zinc deficiency may occur in a soil when the element is unavailable, although abundant. Whereas the element was shown by Alben and Boggs (1936) to be readily available in some soils of fairly low total content, Gilbert (1957) reported that soil reaction, texture and composition greatly affect zinc availability and hence its uptake by the plant.

Some factors that influence the absorption of zinc by various plants, in natural soil and synthetic media, have been subject to investigations for more than 30 years. Hoagland and his associates (1936) suggested that zinc possibly undergoes transformations that are brought about by microorganisms and which have effects on availability. This idea was confirmed by Starkey (1955) who stated that microorganisms modify the trace elements provided to the plants, and affect their availability to plants by assimilating them, by oxidation and reduction, by precipitation and solution and by production of organic complexes of the metals and decomposition of these complexes.

There is enough evidence at hand to consider the pH value as one of the essential factors that influence zinc availability in the soil and its absorption by the plants. Floyd (1917) observed some cases of injury to citrus trees apparently induced by ground limestone. Many years later, Alben and Boggs (1936) found that zinc deficiency in citrus and pecan had resulted because of overliming. Hibbard (1940) concluded that zinc seems to be quite unavailable to some species of plants in strongly alkaline soils although the total amount present may be adequate. Lott (1938) found also that zinc toxicity may be reduced by raising the pH of the soil. This seems to be an indirect effect due to reduction in zinc absorption. Mitchell (1954) noticed that liming to the recommended level does not seem to reduce zinc uptake. This may be true in acid soils, because, disregarding interference by other factors, zinc becomes most available at pH values between 5.5 and 7.0, and drops sharply in availability below pH 5.0 and above pH 7.5 as found by Truog (1948, 1951). Thus, zinc can be deficient both under strongly acid and highly alkaline conditions. Although zinc is leached easily under very acid conditions and is thus lost, but as the pH rises its absorption is reduced until the point of unavailability is reached. Viets (1966) observed that zinc deficiency was most prevalent on neutral-to-alkaline soils containing lime in the profile, it was also common on acidic leached soils.

In a pot experiment performed by Wear (1956) to study the effect of $CaCO_3$, $CaSO_4$ and Na_2CO_3 on the uptake of zinc by sorghum grown in a zinc deficient sandy loam, application of $CaCO_3$ at 2000 lb/acre reduced zinc uptake, increased the soil pH from 5.7 to 6.6 and the content of plant from 0.78 to 1.09%. Increased raise of Na_2CO_3 also reduced the zinc uptake and increased the pH of the soil without increasing its Ca content. On the other hand 2000 lb/acre $CaSO_4$ decreased the soil pH from 5.6 to 4.8 and increased zinc uptake and the Ca content of the plant from 0.78 to 1.01%. Thus, it is clear that reduction in zinc uptake was due to a pH effect and not to a Ca effect. In another experiment, Churbanov (1959) found that liming decreased Zn^{65} uptake by mustard plants in podzoic sandy soil, the zinc content decreased in the order: leaves > stem > pod valves > seeds.

In the fungi, this situation is reinforced by the findings of Schneider and Siegel (1958): application of $CaCO_3$ at levels as low as 2% decreased zinc uptake by Aspergillus niger van Tieghem, in calcareous soil, and complete inhibition of uptake occurred with addition of 20% lime. The decrease in zinc availability to Aspergillus niger van Tieghem, is due to increasing pH from 3.5 to 5.0. Complete unavailability being reached at pH above 6.0. There was no evidence of increased zinc availability by the formation of zincate at pH above 8.0.

On the enzymatic level, it was found by Hoch and Vallee (1958) that the exposure of yeast alcohol dehydrogenase to pH levels below 6.0 resulted in a rapid loss of activity. The loss of activity was directly proportional to the removal of intrinsic zinc from the enzyme. Thus, on dialysis, 50% of intrinsic zinc and activity was lost at pH 5.0 and all zinc and activity at pH 4.5.

The ionic interaction between zinc and other elements has been extensively studied. The effect of phophorus on zinc absorption and zinc content of plants was studied by many investigators. In greenhouse experiments with Red kidney beans, Phasoelus vulgaris L., Burleson et al. (1961) found that severe phosphorus-induced zinc deficiency occurred with phosphorus fertilization. Mutual antagonism was clear between the two elements, when both zinc and phosphorus were applied, the uptake of both zinc and phosphorus were reduced. In an earlier report, Boawn and his co-workers (1954) found that application of phosphorus had no effect on the uptake of either applied or native soil zinc. Doubling the concentration of phosphorus in the plant tissues failed to produce zinc deficiency symptoms or to reduce the yield of dry matter of beans. The authors stated that the amount of extractable phosphate in the soil was not related to the appearance of zinc deficiency symptoms. These results contradict what has been found later in many experiments.

In greenhouse experiments performed by Ellis et al. (1964) on field beans and corn, it was noticed that the application of 437 and 655 pounds of phosphorus per acre decreased the zinc concentration in the plants. In most experiments a negative correlation was noted between zinc and phosphorus concentrations in the plant tissues. The data obtained indicated a mutual antagonism between phosphorus and zinc in their uptake and accumulation in the plant. Labanauskas et al. (1958) found that the leaves of avocado trees, receiving 20 lb/tree treble superphosphate in two consecutive years contained appreciability lower concentrations of zinc and copper than those from trees receiving no phosphorus. Leggett and Boawn (1963) gave a good review of the range of crops and conditions where phosphorus applications have brought about or intensified zinc deficiency symptoms. Boawn and Leggett (1964), working with potatoes grown on field plots and in nutrient solutions at levels of zinc and phosphorus nutrition that would produce plants ranging from no zinc deficiency to severe zinc deficiency, found that increasing the supply of phosphorus induced a growth disorder that could be eliminated by an increased supply of zinc.

Other greenhouse experiments, with tomato plants, were carried out by Martin et al. (1965) to study the influence of

temperature upon occurrence of phosphorus-induced zinc-deficiency. Results on soil with moderately low (0.9 ppm) dithizone extractable zinc showed phosphorus to induce zinc-deficiency symptoms at 50° and 60°F. but, not at 70° and 80° F. In soil from an acutely zinc deficient area (0.1 ppm of zinc), phosphorus-induced zinc-deficiency was observed at all temperatures. Phosphorus applications were observed to reduce zinc concentration in the tissue, while zinc application tended to reduce phosphorus concentration. Stunted plants, showing acute zinc-deficiency symptoms, had about the same zinc and phosphorus concentration on the dry weight basis, as did normal appearing plants grown on higher soil temperature with the same phosphorus treatment. Phosphorus-induced zinc-deficiencies in field experiments were also observed by Martin et al. in field corn (1958) and in sweet corn, tomato and field corn (1959).

The effects of other elements, in the nutrient solutions, on the availability and absorption of zinc, were studied by Fuehring and Soofi (1964), who noticed a highly significant positive interaction between zinc and manganese on the grain yield of corn. Increasing either element decreased the toxic effect of the other. Malavolta and his associates (1956), working on coffee plants, Coffea arabica L., found that iron concentrations in the nutrient solution did not affect the uptake of Zn^{65}, but raising the level of manganese, copper and

molybdenum from 1.0 to 10.0, 0.5 to 5.0, and 0.01 to 1.0 ppm respectively lowered the Zn^{65} absorption 50 % or more. Jurinak and Thorne (1955) studied the solubility of zinc under alkaline conditions in a zinc-bentonite system. With sodium and potassium hydroxides, zinc solubility was lowest between pH 5.5 and 6.7, but increased at higher pH, suggesting the formation of soluble alkali zincate. With calcium hydroxide no increase in zinc solubility was noted from the minimum value at pH 7.6, suggesting the formation of insoluble calcium zincate. Analysis did not reveal the ionic species of zinc in solution. In a report by McHargue and Calfee (1931), on the effects of trace elements on the growth and metabolism of Aspergillus niger van Tiegh., and Rhizopus nigricans Ehr., they found that the uptake of calcium, phosphorus and magnesium was increased by zinc, but the uptake of nitrogen was diminished.

The effect of temperature on the growth of Pole beans, was studied along with phosphorus application, by Apple and Butts (1953). In the greenhouse experiments, the authors found that Pole bean plants, grown at 82°F were significantly higher in phosphorus and dry weight, especially when no phosphorus

was supplied, than those at 63^{0}F. Mack et al. (1964) observed that an increase in soil temperature, from 54^{o} to 78^{0}F, increased the dry weight of snap beans by 60 - 85 %, while dry weights of peas were increased only little or reduced.

The effect of soil temperature on the availability of indigenous soil zinc was studied by Bauer and Lindsay (1965). They used corn, Zeamays L., grown for two weeks in soils having two zinc levels (0.0 and 0.75 ppm of zinc as $ZnSO_4.7H_2O$) which were subjected to various temperatures (5, 17, 31 and 43^{o}C.) for various intervals (0, 1, 3, and 6 weeks). They found that both yield and uptake of zinc were increased from soil previously incubated at 43^{o}C. for 1-3 weeks. The 6-week incubation tended to reduce available zinc over shorter periods.

In general, we can conclude that zinc deficiency is far more common in climates with high light intensity and elevated temperature. Viets (1966) noted that the sunny side of trees showed more zinc deficiency than the shady side. In an earlier report by Skoog (1940), it was mentioned that high light causes destruction of auxin by blue and ultraviolet rays.

The effect of stage of growth on the plant content of zinc was studied by William and Moore (1952). They used Algerian oats, grown on 13 soils, and found that the zinc content of the

whole plant decreased from an initially high value until the flowering stage after which there was little further change. The leaf always had a higher zinc content than the stem and there was an almost linear decrease as the plant matured. In the stem the rate of decrease was less after flowering. The uptake of zinc continued throughout the period of growth. The amount of zinc in the leaf, and also generally that in the stem, increased until flowering and then decreased as translocation to the grain occured.

The translocation of absorbed Zn^{65} in subterranian clover Trifolium subterranium L., was studied by Riceman and Jone (1960). They found that recently absorbed Zn^{65} in normal plants, reached high concentrations in the roots and the main axis, and hardly ever moved from the youngest to the fully expanded older leaves. While in plants recovering from zinc deficiency, Zn^{65} reached high concentrations in all living tissues including the oldest leaves.

Many experiments were designed to study the translocation of elements, when topically applied to the plants. Viets (1951), working on beans and corn, reported that poor growth and browning of the leaves of beans, were improved with sprays of 0.3 % $ZnSO_4 \cdot 7\ H_2O$. The absorption and translocation of Zn^{65} in Pisum sativum L., variety Alaska, were studied by Sudia and Link (1963). They applied two drops of $Zn^{65}Cl_2$ to the two opposite leaflets of the

first bloom node leaf, and found that zinc is translocated from the first bloom-node leaf two days prior to anthsis (full bloom). The preferential accumulation of Zn by the first pod, reaches a maximum about five days after full bloom and the magnitude of this accumulation remained essentially constant as the pod approached maturity. The zinc accumulated by the ovules was found to increase gradually beginning at about the fifth day after full bloom and was accompanied by a decrease in zinc retained by the carpel. Malvolta et al. (1965) noticed that the foliar absroption by Coffea arabica L., of Zn^{65} was 8 times as high as root absorption. Brushing the lower surface of the leaves was the most effective method of foliar application, giving about 40% absorption and 13% translocation to the shoots and roots.

Wallihan and Heymann-Herschberg (1956) found, in citrus, that maximum translocation of zinc occurred when it was applied near the middle of the leaf. Least translocation resulted from application at the leaf margin. When comparing the upper and the lower surfaces of the leaves, they observed no difference in absorption and translocation, this led them to the conclusion that absorption through stomata was unimportant, if it occurred at all.

Wood and Sibley (1950) studied the distribution of zinc in oat plants and reported that zinc was absorbed by the roots of

oat throughout the life cycle of the plant, with as much as 20 to 30 percent of the total zinc in the plant found in the leaves. Sayre (1952) studied the distribution of Zn^{65} in corn leaves and found that this isotops accumulated around the primary veins of the blade.

Foliar and root applications of zinc sulfate were performed on soybean, tomato and corn by Leyden and Toth (1960). Their results showed that $Zn^{65}SO_4$ was absorbed and, to some extent, distributed throughout soybean, tomato and maize plants, growing in sand cultures. More Zn^{65} was absorbed from root application than from similar applications to the leaves of soybean and tomatoes, but more was absorbed by leaves than roots in mays.

III. Materials and Methods

Preparation of the plants

Seeds of Pinto beans, Phaseolus vulgaris L. var Pinto, were washed thoroughly with tap water, soaked in hot water (temperature 50°- 60°C.) for 3 - 4 minutes and chilled with cold water for an equal period. This treatment was repeated many times. (Seeds used in the soil experiment were disinfected with Spergon as dust in the ratio of 3 - 4 oz./100 lb. of seeds.[1]). Clean seeds were then spread upon a layer of wet fine glasswool in enameled pans and kept in the dark for germination. A week later, young seedlings[2] with two opposite primary leaves, each measuring about 15 cm^2, were carefully removed from the germination pans, and their roots gently rinsed successively with tap water and distilled water. The stem of each seedling was inserted in a hole (diameter of 5 mm.) made in a cardboard cover of Drosophila jars (capacity of 250 ml.). Two similar holes were made in each cover for aeration.

---

1. Allard R. W. and Smith F. L. 1954.
2. Seedlings, chosen for the experiments, were of the same age and about the same size. Seedlings that had any visible abnormalities, as having three cotyledons or three primary leaves were discarded.

Clean Drosophila jars[1] were prepared, each containing 150 ml. of the nutrient solution. All the root system of each seedling was submerged in the nutrient solution. The jars were wrapped with black cylinders of cardboard to facilitate the normal growth of the roots. The seedlings used in the experiments were those which had their primary opposite leaves fully expanded, and each leaf having a surface of about 23 cm^2.

Nutrient solutions

The complete nutrient solution used was prepared according to Arnon and Hoagland (1940)[2] as follows:

---

1. In the preliminary experiments, cork plates were used to support the young seedlings in plastic boxes having the nutrient solution (according to Wybenga J. M., 1957, pp 36). This precedure was discontinued in the experiments reported here, due to the diffusion of certain substances which had obviously an inhibitory effect on the growth of the seedlings.

2. Steward F. C., 1963 pp. 100.

Chemical	gm/liter[1]	Molar[2]
KNO_3	1.020	0.010
$Ca(NO_3)_2$	0.492	0.003
$NH_4H_2PO_4$	0.230	0.002
$MgSO_4 \cdot 7H_2O$	0.490	0.002
$FeCl_3$ 0.5%[3]	0.6 ml./liter	
Tartaric acid 0.4%	1.3 X weekly	
	mg/liter	
H_3BO_3	2.86	
$MnCl_2 \cdot 4H_2O$	1.81	
$CuSO_4 \cdot 5H_2O$	0.08	
$ZnSO_4 7H_2O$	0.22	
$H_2MoO_4 \cdot H_2O$ ($MoO_3 + H_2O$)	0.09	

This solution is reported to have a pH of 4.5 - 6.0, and an osmotic pressure approximately 0.6 - 0.7 atmosphere[2]. The nutrient solution prepared according to the above formula had a pH of 4.7 $\pm$ 0.1.

Radioactive carrier-free zinc, in the form of $Zn^{65}Cl_2$[4], was used as a tracer, this isotope had a radioactivity of 7 ucu/ml.

---

1. Steward F. C., 1963 pp. 100.
2. Hewitt E. J., 1966.
3. $FeSO_4 \cdot 7H_2O$ was replaced by $FeCl_3$.
4. Obtained from: Radiochemical Center, Amersham, England.

Aeration of nutrient solutions

An air pump (Model: Sargent 0406 V2-154 pressure pump), with rubber and glass tubing connections, was used to aerate the nutrient solutions for 10-15 minutes daily. The terminal parts were capillary tubes, each of which was inserted through a hole in the cardboard cover of the nutrient jar. A second hole was left open to release excess air bubled into the nutrient soluttion. By this set-up 8 jars were aerated at one time.

Aeration was substituted by renewal of the nutrient solution during the proper experimentation period, to eliminate any possible root injury caused by air bubbles, to restore the original concentration of nutrients and to avoid introducing excess amounts of atmospheric gases that may be involved in chemical reactions within the solution or affect the rate of absorption by the roots.

Zinc-65 Assay

To avoid any diurnal effect, all experiments, unless stated otherwise, were started at noon and the plants were harvested at noon, at the end of the time designated to each experiment. The normal temperature of the laboratory was 25 $\pm$ 2^{o}C.

At the end of each experiment, the plants were removed from their jars; the lower part of the stem and roots of each plant were thoroughly rinsed under running tap water for 3 - 4 minutes and then with distilled water. After plotting the plants on clean filter papers, each was divided into roots[1], stem and leaves. The plants were stratified between plotting sheets in enameled pans and oven dried (at 50°C. for 48 hours). each part of the plant body was thoroughly ground, weighed on a sensitive balance[2] and kept separately in a clean test tube for radioactive zinc assay[3]. Dry weight was used in all experiments.

A well-type scintillation counter[4] was used for assay, it had an efficiency of about 49.3%[5] and a background of 160 ± 10 counts per minute. Radioactivity in the plants were measured as cpm.

---

1. Any adventitious roots on the stem, were removed and added to the root part of the divided plant.
2. Mikrowa balance type AW-10.
3. Gamma counting is preferred, since there is relatively low self-absorption of gamma rays, complicated separation may be avoided and the tissues can be counted directly or in solution. (Francis G. E., Mulligan W. and Wormall A., 1959, pp. 470, Comar C.L., 1955, pp. 319).
4. Chicago-Nuclear, model 161A, Chicago, U. S. A.
5. Since positrons and low-energy x-ray resulting from electron capture will be almost completely absorbed, in the crystal housing, only 49.3% of disintegration is detected by the scintillation counter (Wang C.H. and Willis D. L., 1955 pp. 354).

Depletion factor was calculated in each case as follows:

$$\frac{\text{Initial cpm/1 ml. Ext. Sol. - Final cpm/1 ml. Ext. Sol.}}{\text{dry weight of plant meterial}} \times 100$$

to give depletion per 100 mg. of plant body on dry weight basis.

Autoradiograms were prepared by pressing mounted dry plant material against Kodak Blue Brand (BB-54) x-ray films. The plants and the x-ray films were kept in contact in light-proof cassettes for 15 days. At the end of the exposure period, the x-ray films were developed by Red liquamat Developer-replenisher[1] and fixed by Liquamat Fixer-replenisher[1].

Absorption of Zn^{65} from different solutions

Four groups of plants were grown in different solutions for 24 hours. Deionized water was used in the preparation of the first solution. Distilled water was used in the second solution. The third was a nutrient solution the pH of which was adjusted to 6.8. The fourth was a nutrient solution that had a pH of 4.95. Water used in all solutions was boiled and cooled before preparation of each solution. All the plants were treated with 0.01 M $CaCl_2$ for 1 hour at the end of each experiment.

---

1. Obtained from: GAF - Anscofilms Company, U. S. A.

Calcium chloride treatment

All the plants used in this experiment were kept in the nutrient solution having Zn^{65} for 24 hours. At the end of the experiment the plants were rinsed and divided into seven groups. The roots were immersed in 0.01 M $CaCl_2$ for various intervals: 0, instantaneous, 5, 10, 20, 40 and 60 minutes. This experiment was disigned to study the significance of $CaCl_2$ treatment which is used for the removal of zinc from the "free space" in the roots.

Time-course absorption

The plants used in this experiment were kept in the nutrient solution containing Zn^{65} for various time intervals: 2, 4, 8, 16, 32, 64, 96 and 128 minutes. The experiment was performed to study the possibility of obtaining maximal absorption of zinc within a limited short time.

Determination of zinc absorption by stable zinc variation

A constant concentration of $Zn^{65}Cl_2$ was used in this experiment (14 ucu/liter), but the concentration of the stable isotope in the nutrient solution was varied. The concentrations used were: 0.0, 0.05, 0.25, 2.50, 25.0, and 50 ppm of zinc/liter.

Zn^{65} concentration

In this experiment various concentrations of $Zn^{65}Cl_2$ were used, both in nutrient solution and in distilled water. The final pH of the experimental solutions was not adjusted to 6.8.

Previous treatment with stable zinc

Plants for this experiment were grown in nutrient solution having different concentrations of zinc, for one week, after which the plants were transferred to nutrient solution containing $Zn^{65}Cl_2$ in the amount of 14 ucu/liter, for 24 and 48 hours.

Experiments for optimum pH determination

In these experiments the pH of the nutrient solution was adjusted[1] to different levels by the addition of appropriate amounts of 0.1 N citric acid for pH 4.0, and appropriate amounts of 0.1 N potassium hydroxide for higher pH levels. Once the optimum pH range for Zn^{65} absorption was established, as will be discussed in the chapter on results and discussion, the pH of the nutrient solution for all the subsequent experiments was adjusted to 6.8.

---

1. With a pH meter 22 Type PHM22q, Radiometer - Copenhagen.

For the optimum pH determination, 3 experiments were performed; the first was concerned with estimating the approximate pH for the highest zinc absorption during 24 hours, the second for the pH level for the highest zinc absorption during 48 hours, and the third was carried out to narrow down the optimum pH level for maximum Zn^{65} absoprtion. The pH levels used in these experiments were: 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 in the first and the second experiments, and 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, and 7.4 in the third experiment.

Soil pH experiment

Four soil samples were collected from different areas of Lebanon. The pH of each sample was determined, and a fifth sample was prepared by adjusting the pH of the AUB soil to pH 7.0, by thoroughly mixing appropriate amount of 0.01 N sulfuric acid. The soil samples were mixed with vermiculite and kept in 5 clay pots, each having a top diameter of 12.5 cm. Seeds of pinto beans were disinfected with Spergon and 7 seeds planted in each pot. The seedlings were watered with distilled water till they reached approximately the same size of seedlings used in nutrient solution experiments. At this stage of growth, they were watered with distilled water having $Zn^{65}Cl_2$ for three days, each pot received 100 ml. of the radioactive solution daily. On the fourth day,

they were given only distilled water. $Zn^{65}Cl_2$ added to the distilled water had an activity of 14 ucu/liter.

Light experiments

In these experiments three groups of plants were subjected to different lighting conditions, one group was illuminated for 24 hours by 3 fluorescent tubes[1]. This light source provided a light intensity of about 3000 lumens at the height of the primary leaves. A second group was kept under natural indoor light conditions for 24 hours (day light and night darkness). The third group was kept in complete darkness for 24 hours.

In another experiment, a group of plants was kept in complete darkness for 48 hours, a second group of plants was kept under normal laboratory lighting conditions (day and night), one of the primary leaves of each plant in this group was completely shielded with black sheets of cardboard, but the opposite leaf was left uncovered as a control.

Diurnal variation

Thirty six plants were divided into six groups, each group was subjected to 4 hour illumination period, using fluorescent tubes which provided light intensity of 3000 lumens at the height

---

1. Claude: deluke day light, each 20 watt.

of the primary leaves. The experiment started at 10:00 a.m. and ended at 10:00 a.m. the next day.

Temperature experiment

18 plants were divided into 3 groups, the first group was kept at 5°C. for 24 hours, the second at 15°C. and the third at 25° ± 2°C. for 24 hours. All plants were given the same concentration of $Zn^{65}Cl_2$, 14 ucu/liter.

Age experiment

Plants of different ages were used in this experiment. Plants of the first group were one week old (after emergence), the primary leaves were not completely expanded, and the apical bud was very small. The second group was two weeks old, with fully expanded larger primary leaves and the apical bud is slightly elongated. The plants of the third group, in addition to the well developed primary leaves, each had a small fully expanded first compound leaf (0.5 cm. long tripartite leaflets). The plants of the fourth group were four weeks old, having large well developed primary leaves and two compound leaves, the third

compound leaf had not yet developed normally in any plant.

Translocation experiments

Translocation of Zn^{65} within the plant was studied by the following methods:

A. By keeping 24 plants in nutrient solution containing 14 ucu of Zm^{65}/liter for 24 hours. All the plants were removed from the radioactive solution, their roots and the lower part of the stem were thoroughly rinsed under running tap water and then with distilled water. They were divided into 4 groups. The first group was treated for Zn^{65} assay directly. The second group was kept in nutrient solution for 24 hours, the third group was kept for 48 hours in the nutrient solution and the fourth was kept for 72 hours, after which they were oven dried and assayed for radioactive zinc.

B. By growing the seedlings in a nutrient solution which contained radioactive Zn^{65}, 14 ucu/liter, for one week. At the end of the exposure period the plants were mounted and used for autoradiography.

C. Using disposable syringes with JB 25 needles, 0.1 ml. of $Zn^{65}Cl_2$ solution, having 0.7 ucu of radioactivity, was injected into different parts of the plant body. In one plant, injection was made into th petiole of one primary leaf; in a second, injection was made into the stem just below the bases of the opposite

primary leaves; in a third plant, injection was made into the middle of the stem near the scars of the cotyledons; in a forth plant, injection was made into the base of the stem just above the roots and in a fifth plant, injection was made in between the bases of the two opposite primary leaves. All the plants were kept in the nutrient solution for 24 hours, then mounted and used for autoradiography.

D. Foliar application was performed by placing a drop of $Zn^{65}Cl_2$ solution, having 0.35 ucu Zn^{65}/liter, on the upper surface of one primary leaf of the plant, In one case the drop was applied to the tip of the blade, in a second case it was applied to the base of the blade. In a second experiment, the upper surface of one primary leaf was brushed with $Zn^{65}Cl_2$ solution having 2 ucu of Zn^{65}, and the surface of one primary leaf of another plant was brushed with an equal amount of the radioactive zinc chloride. The plants of the two experiments were kept in the nutrient solution for 24 hours. At the end of the exposure period they were assayed for zinc by the scintillation counter.

IV. RESULTS AND DISCUSSION

Absorption in relation to the nature of the solution.

Table 1 shows that the highest quantities of Zn^{65} were absorbed by Pinto bean seedlings grown in a solution that contained nothing but $Zn^{65}Cl_2$. It is evident from Table 1 that the maximum absorption of Zn^{65} occurred from solutions prepared by adding $Zn^{65}Cl_2$ to deionized water. The plants grown in this solution for 24 hours absorbed 87221 cpm/100 mg of dry weight. When normal distilled water was used in preparing the solution, about 70 % of the above amount was absorbed by the plants. When the plants were grown in a complete nutrient solution at pH 6.8, a drastic decrease in Zn^{65} absorption was noticed. The plants could absorb 7442 cpm/100 mg of dry weight, which is about 8.5% of the highest amount absorbed by plants grown in a solution prepared by deionized water. The plants grown in a complete nutrient solution having a pH of 4.95, absorbed 4408 cpm/100 mg of dry weight, which is about 5% of the highest absorption of Zn^{65} obtained in the experiment. Thus, the competition of other cations or the lower pH, in the acidic side of the scale, reduced Zn^{65} absorption.

Translocation of Zn^{65} to the shoot was favored by the above factors, namely the presence of other cations or the low

Table 1

"Absorption and translocation of Zn^{65} from different solutions* for a standard period of 24 hours"

Solution	pH	cpm / 100 mg dry weight					Depletion	root leaves R/L	stem leaves S/L	percent translocation to shoot
		leaves	stem	shoot	root	whole plant				
Deionized water	6.35	7240	23851	15546	230572	87221	335	30	3	12.6
Distilled water	6.10	5264	18827	11746	159054	60848	348	30+	8+	13.8
Nutrient solution	6.8	196	993	595	21338	7442	254	108	21	5.4
Nutrient solution	4.95	438	1085	762	11700	4408	104	26	10+	12.2

* Water was boiled and cooled before preparing each solution.

pH of the solution. Maximum translocation occurred in plants grown in solutions containing other cations besides Zn^{++} or in solutions having lower pH values.

An increase in translocation at acidic pH levels was confirmed also by pH experiments as will be discussed later.

The importance of $CaCl_2$ treatment.

Treatment with 0.01 M $CaCl_2$ for various intervals of time showed no significant decrease in the estimated Zn^{65} absorbed by the plants. Wallace and co- workers (1963) used 0.01 M $CaCl_2$ for one hour to remove monovalent cations from the "free-space" in the root. This treatment was applied, in this investigation, for regular intervals to Pinto bean roots at the end of the absorption experiment. The results are presented in Table 2. There seems to be no reliable results indicating the importance of this treatment in the case of zinc absorption. The only marked change was noticed in the translocated zinc.

The plants which were not treated with $CaCl_2$, or received an instantaneous treatment, showed a higher percentage of translocation to the stem and the leaves. This may suggest that Ca^{++} ions do not only replace Zn^{++} in the "free-space" but they probably interfere with the already translocated zinc.

Table 2

"Effect of treatment with 0.01 M $CaCl_2$ on the absorption and translocation of Zn-65"

$CaCl_2$ Duration "Minutes"	Leaves	Stem	Root	Whole plant	Root/ Leaves	Stem/ Leaves	Percent translocation
0	120	669	25855	8881	215	5.6	3
Instentaneous	141	688	20854	7228	148	4.9	3.9
5	62	355	23334	7917	376	5.7	1.8
10	69	502	25471	8681	369	7.3	2.2
20	33	367	24699	8366	748	11.1	1.6
40	107	301	19265	6558	180	2.8	2.0
60	62	388	21447	7299	346	6.2	2.0

The importance of $CaCl_2$ in absorption experiments needs more investigations to evaluate its use in such experiments, espacially when bivalent cations are involved.

Absorption in relation to time of treatment.

Figure 1 and Table 3 represant the results obtained in an experiment to determine whether the root reaches a state of "saturation" with zinc or at least if there will be a decrease in the rate of absorption. The results obtained, for intervals ranging from 2 - 128 minutes, show that with time more Zn^{65} was absorbed and the increase is not related only to translocation since the amounts absorbed and accumulated in the root were very high if compared with the amounts translocated to the shoot. This situation was supported also in pH experiments when the absorption was studied for 24 and 48 hours. With time, the absorbed amount of Zn^{65} increased and consequently, there was an increase in translocation to the shoot.

Zn^{65} absorption in 24 hours.

A variation of stable zinc concentration was used with a constant $Zn^{65}Cl_2$ concentration, in order to determine the amount

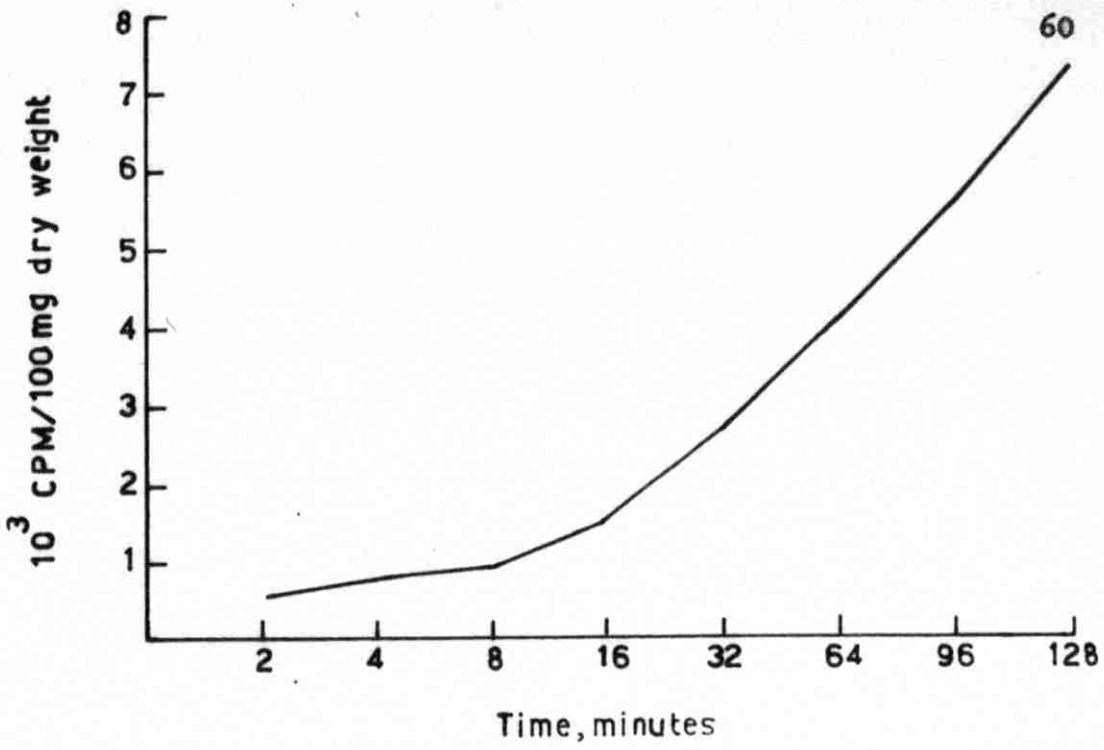

Figure 1. Time – course of Zn^{65} absorption by pinto bean seedlings

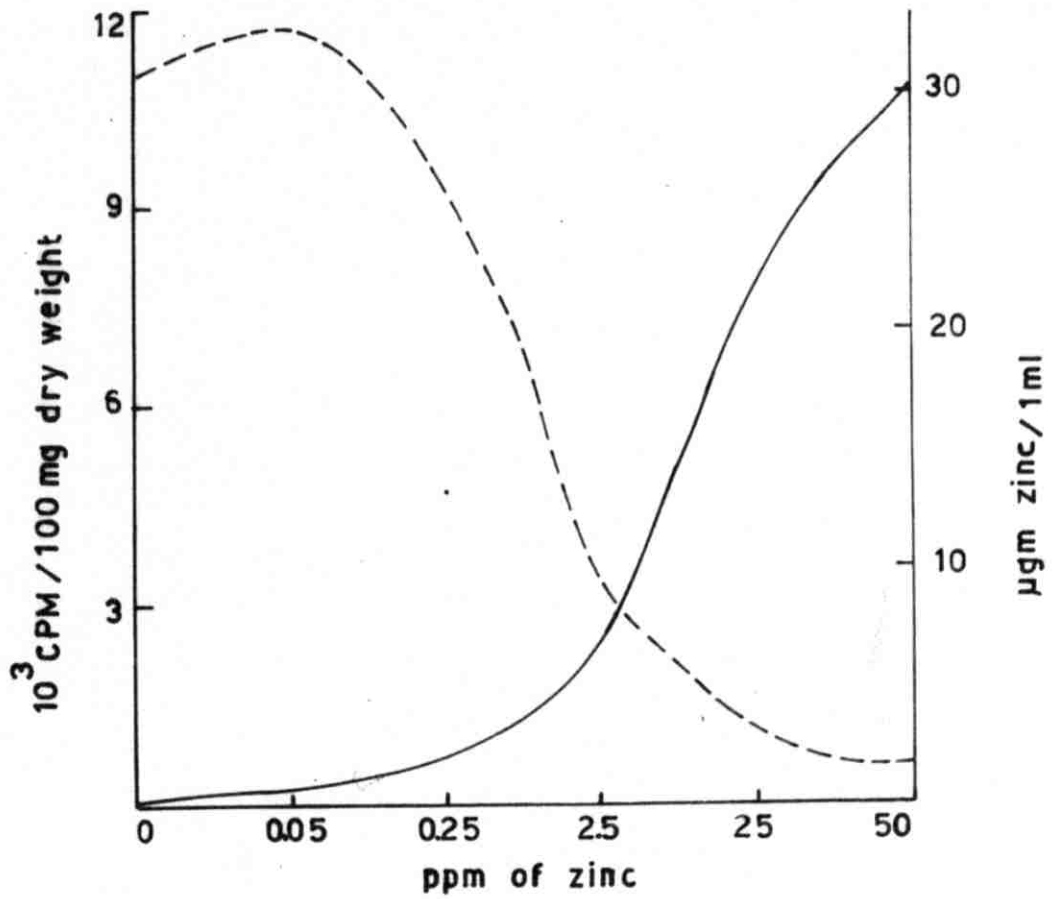

Figure 2. Zinc absorption by the whole plant of pinto bean seedlings in 24 hours.

Table 3

Time-course of Zn^{65} absorption by Pinto bean seedlings. Treated with 0.01 M $CaCl_2$ for one hour.

Time	cpm/100 mg dry weight				Root/	Root/
minutes	Leaves	Stem	Root	Whole plant	Stem	Leaves
2	5	115	1699	606	15	340
4	36	78	2129	748	26	59
8	41	108	2611	937	24	63
16	14	119	4581	1571	39	327
32	41	208	8190	2813	39	199
64	61	411	12388	4287	30	203
96	32	360	16941	5778	47	529
128	48	341	22356	7580	65	466

absorbed from each ml. of nutrient solution. As indicated in Figure 2 and Table 4, the higher the concentration of the stable isotope in the nutrient solution the less radioactive znc-65 was absorbed. The amounts absorbed, in ugm/1 ml. of the nutrient solution, were calculated and showed a direct correlation between the amounts of bothe isotopes absorbed. As expected, the amount of Zn^{65} absorbed was inversely proportional to the concentration of the stable zinc.

Effect of Zn^{65} concentration.

Figure 3 and Table 5 represent the results obtained in an experiment designed to study the effect of concentration. These showed that the rates of absorption and translocation of Zn^{65} were greatly influenced by the concentration of the element in the nutrient solution. Other factors being constant, it was noticed that with an increase in the zinc concentration, there was always a higher accumulation of the element in the plants.

An increase in absorption occurred from the complete nutrient solution as well as from a solution prepared by adding $Zn^{65}Cl_2$ to distilled water, although the absorption of Zn^{65} from distilled water was much more than absorption from the nutrient solution.

Effect of previous treatment with zinc.

When the plants were grown for one week in the nutrient

Table 4

"Average zinc absorption by Pinto bean seedlings in 24 hours." A variation of stable zinc concentration with constant Zn^{65} concentration in the nutrient solution. The pH of all solutions was adjusted to 6.8.

Zince Concentration ppm	cpm/100 mg dry weight				Depletion	ugm/1 ml Absorbed Zinc
	Leaves	Stem	Root	Whole plant		
0.00	211	1592	30831	10878	164.4	0.00
0.05	217	1592	34004	11869	105.6	0.44
0.25	266	1194	26123	9194	84.4	1.84
2.50	120	611	9510	3414	69.6	6.61
25.0	98	287	3134	1173	35.7	22.3
50.0	96	243	2112	817	33.5	30.1

solution to which different concentrations of stable zinc were added, and then grown for 24 hours in a nutrient solution containing Zn^{65} there was a clear effect of the previous treatment on the absorption of Zn^{65}.

As shown in figures 4 and 5 and Table 6, the highest absorption of Zn^{65} occurred when the plants were grown for one week in a nutrient solution having 2 ppm of stable zinc. But, the highest amount of Zn^{65} translocated to the leaves was in the plants grown at lower concentrations of stable zinc. The highest translocation of Zn^{65} to the primary and tripartite leaves did not occur in the plants which were grown in the lowest stable zinc concentration (0.1 ppm of zinc). This indicates that the optimum concentration of zinc is higher than the values suggested by Hewitt (1966) and others.

In a second experiment, other concentrations of stable zinc were used in the nutrient solution and the experimental plants were treated with radioactive zinc for 48 hours. The results of this experiment are presented in Figures 6 and 7 and Table 7.

The highest absorption of Zn^{65} occurred by plants grown for one week in a nutrient solution containing 2.5 ppm of zinc. When the concentration was increased to 25 ppm of zinc, there was

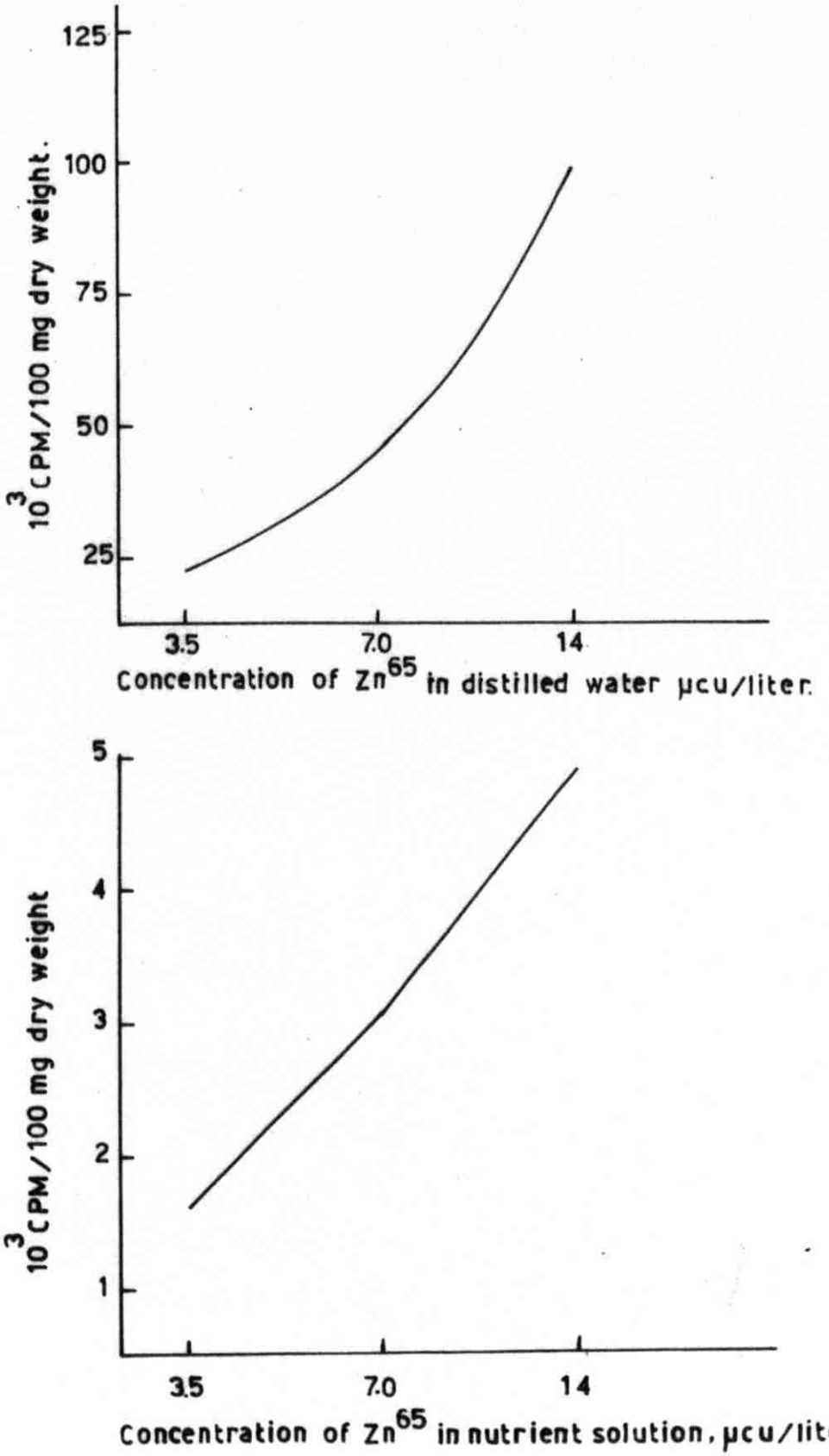

Figure 3. Effect of concentration of Zn^{65} in distilled water and nutrient solution on its absoption by pinto bean seedlings.

Table 5

"effect of various concentrations of Zn^{65} on the absorption and translocation of the element by pinto bean seedlings in 24 hours"

Concentration ucu/liter	pH	cpm/100 mg dry weight				Depletion cpm/1 ml/100 mg. dry weight
		leaves	stem	root	whole plant	
Distilled water						
14	5.50	222	8707	292425	100451	571
7	5.20	205	4093	129219	44506	248
3.5	5.10	142	1694	70302	24046	102
Nutrient solution						
14	4.5	54	731	13557	4781	80
7	4.6	37	372	8978	3129	19
3.5	4.6	25	213	4572	1603	20

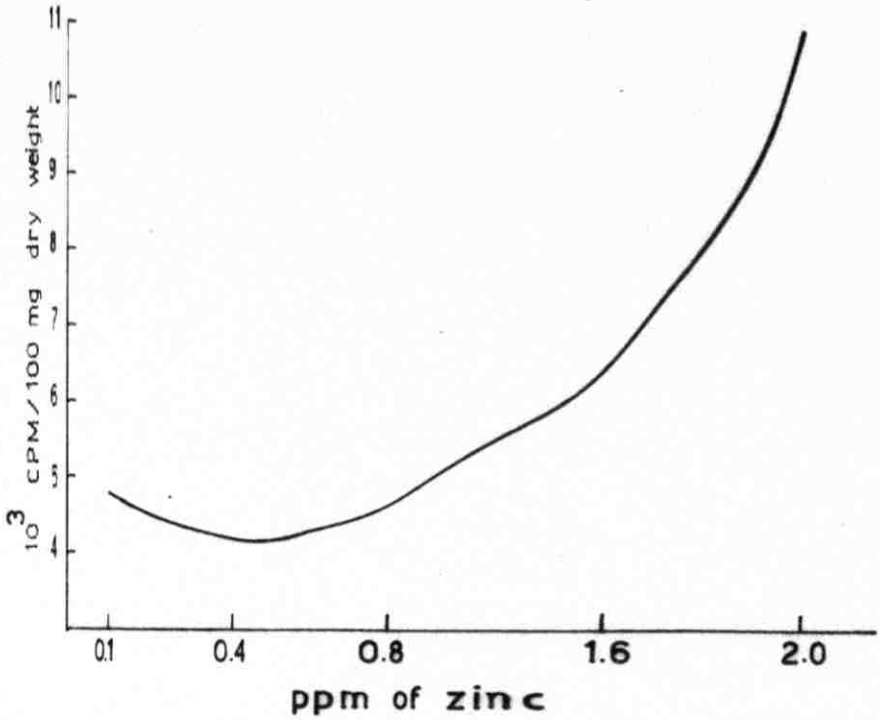

Figure 4. Effect of previous treatment with zinc for one week on the absorption of Zn^{65} in 24 hrs.

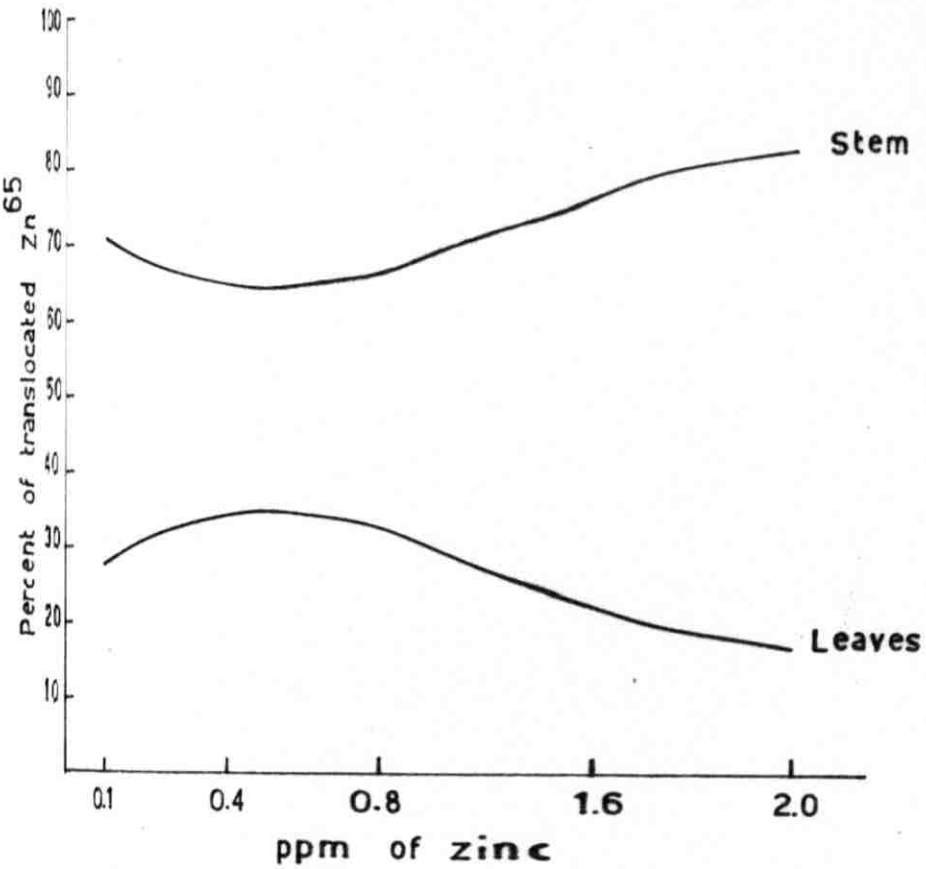

Figure 5. Distribution of Zn^{65} in the shoot of plants previously treated with various concentrations of zinc

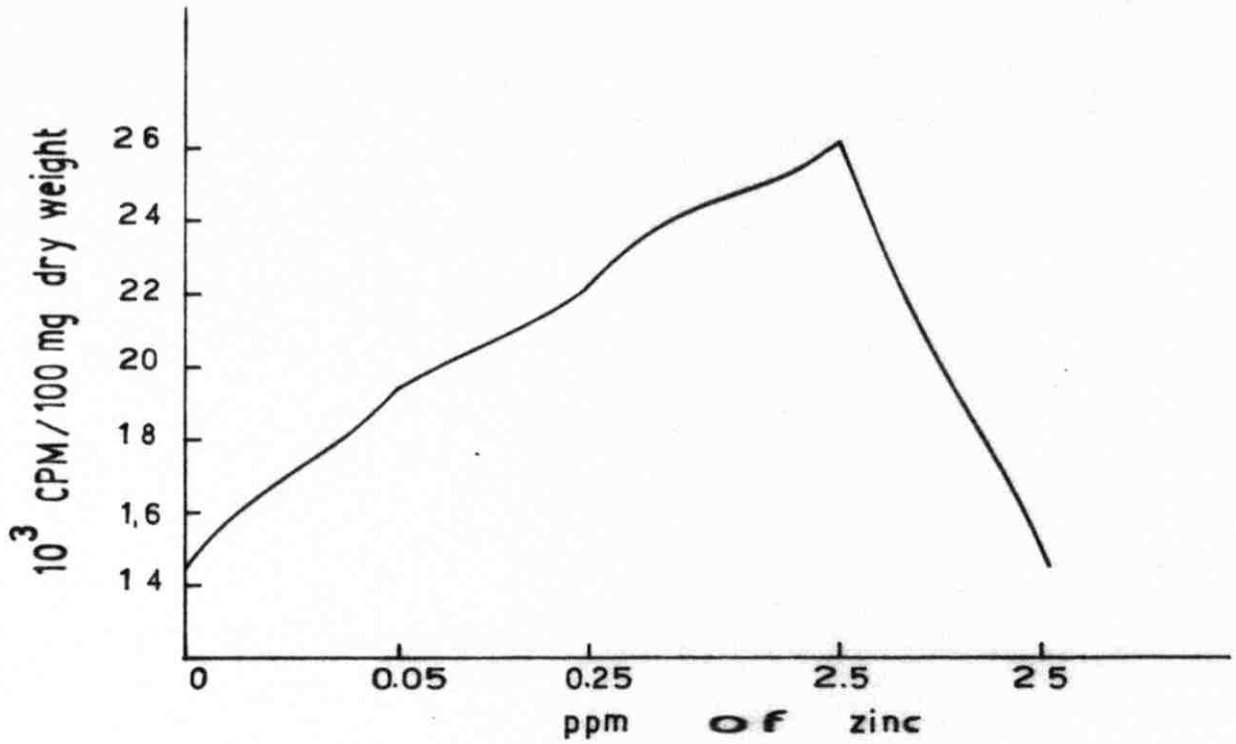

Figure 6. Effect of previous treatment with zinc for one week on Zn^{65} absorption in 48 hrs

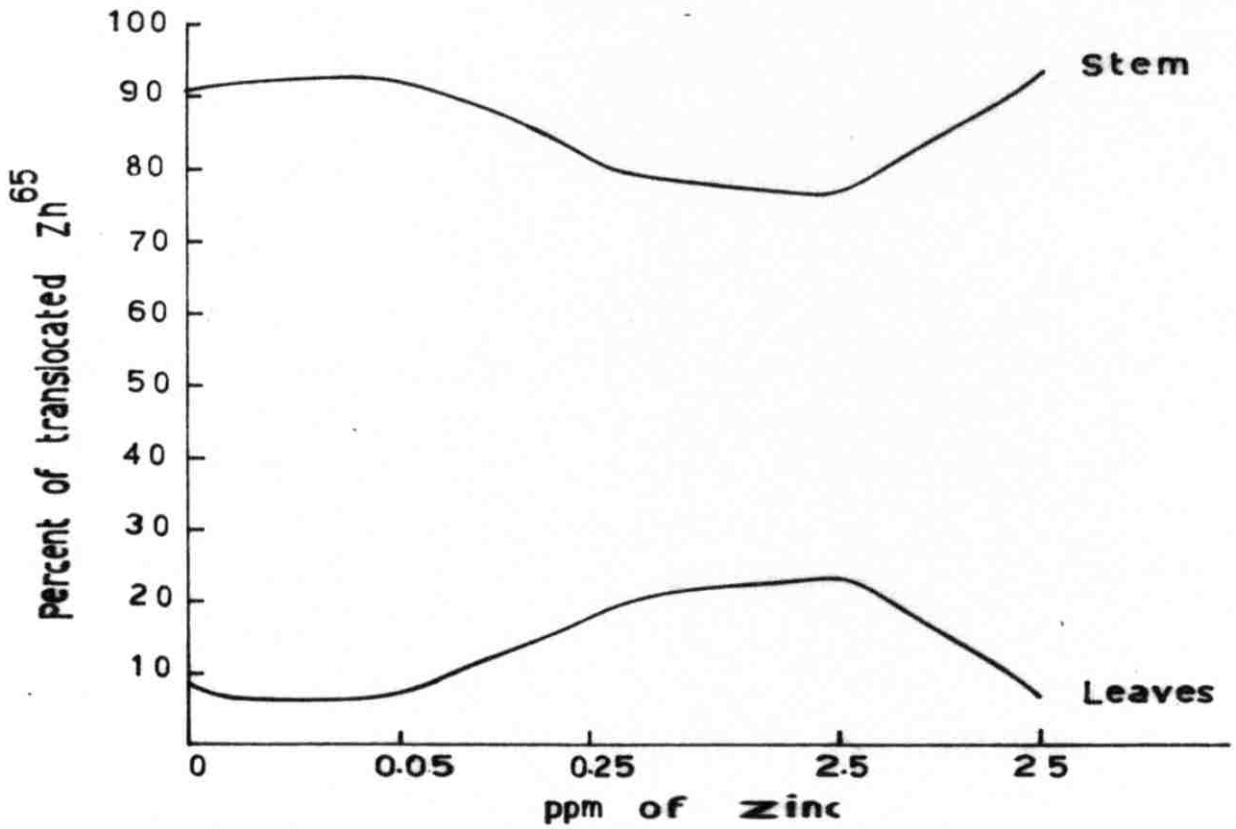

Figure 7. Distribution of Zn^{65} in the shoot of plants previously treated with various concentrations of zinc

Table 6

"Effect of previous treatment with stable zinc on the absorption and translocation of Zn^{65} in 24 hours"

Zinc concentration ppm	cpm/100 mg dry weight					Percent Translocation	
	Primary leaves	compound leaves	Stem	Root	Whole plant	Leaves	Stem
0.1	102	232	830	18226	4848	28.7	71.3
0.4	117	390	959	15279	4186	34.6	65.4
0.8	177	180	724	17212	4573	33.0	67.0
1.6	162	174	1208	24829	6593	21.8	78.2
2.0	81	236	1509	42268	10999	17.3	82.7

Table 7

Effect of previous treatment with stable zinc on the absorption and translocation of Zn^{65} in pinto bean plants within 48 hours.

Zinc concentration ppm	cpm / 100 mg dry weight					depletion cpm/1 ml/ 100 mg dry weight	percent translocation	
	primary leaves	compound leaves	stem	root	whole plant		leaves	stem
0.0	39	98	1432	56905	14618	149	8.7	91.3
0.05	39	162	2126	76431	19689	117	7.4	92.6
0.25	236	365	2606	85175	22095	135	18.7	81.3
2.5	300	444	2441	101842	26232	133	23.4	76.6
25.0	40	40	1282	58410	14943	83	5.9	94.1

a marked decrease in Zn^{65} absorption. From the obtained data, one can suggest that the optimum range for Zn^{65} absorption probably lies between 0.25 and 2.5 ppm of zinc. The highest translocation also took place when 2.5 ppm of stable zinc were used in the nutrient solution.

Optimum pH value for maximal Zn^{65} absorption.

Figures 8 and 9 and Table 8 represent the data obtained in wide range pH experiments for 24 and 48 hours. The results show that the optimum pH of the nutrient solution, for the maximal Zn^{65} absorption, lies around the neutral side of the scale. A slightly acidic solution favored zinc absorption more than slightly alkaline solution. The depletion factor, calculated for the nutrient solutions at various pH values, confirmed the same results. The highest depletion, in 24 and 48 hours, occurred at pH 7.0.

Figure 10 and Table 9 represent the results obtained in experiments carried out to study narrower ranges of pH, between 6.2 and 7.4. It was found that the highest absorption of Zn^{65} was at pH 7.0, but the highest depletion occurred at pH 6.8.

Truog (1948, 1951) mentioned that zinc availability to the plants drops sharply below pH 5.0 and above 7.5. In the present investigation it was found that the availability of Zn^{65} for Pinto bean seedlings, in the nutrient solution, decreased drastically

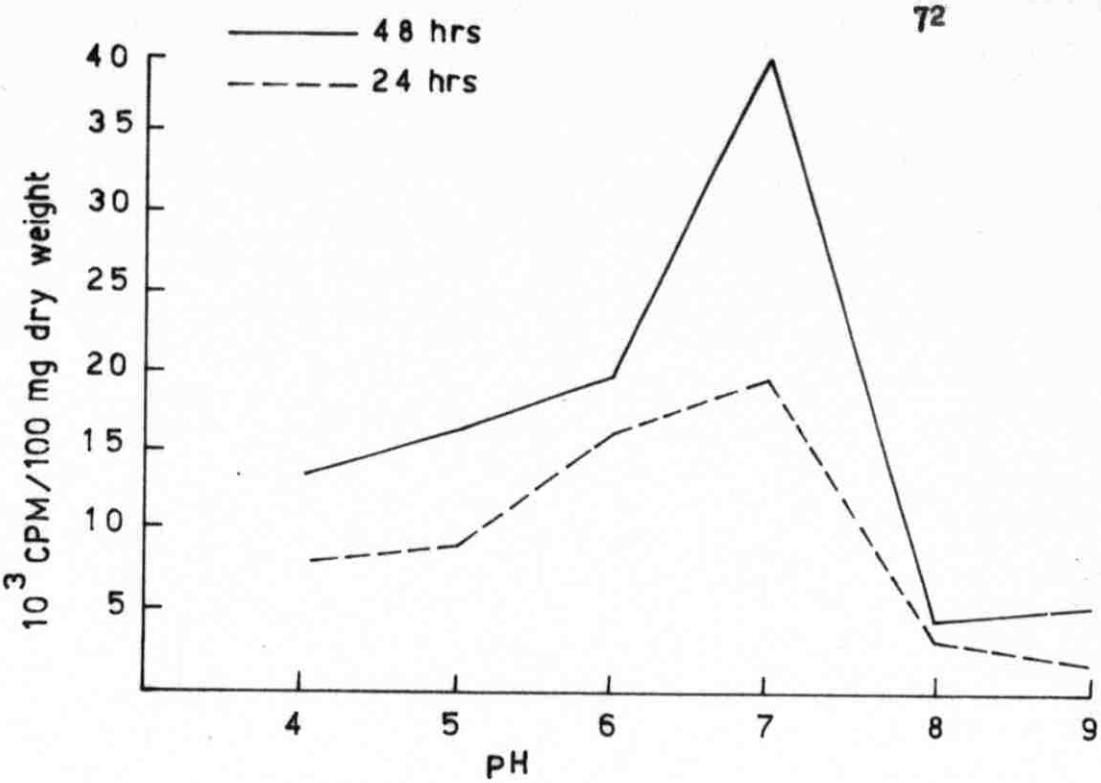

Figure 8. Average Zn^{65} absorption by the whole plants in 24 and 48 hrs

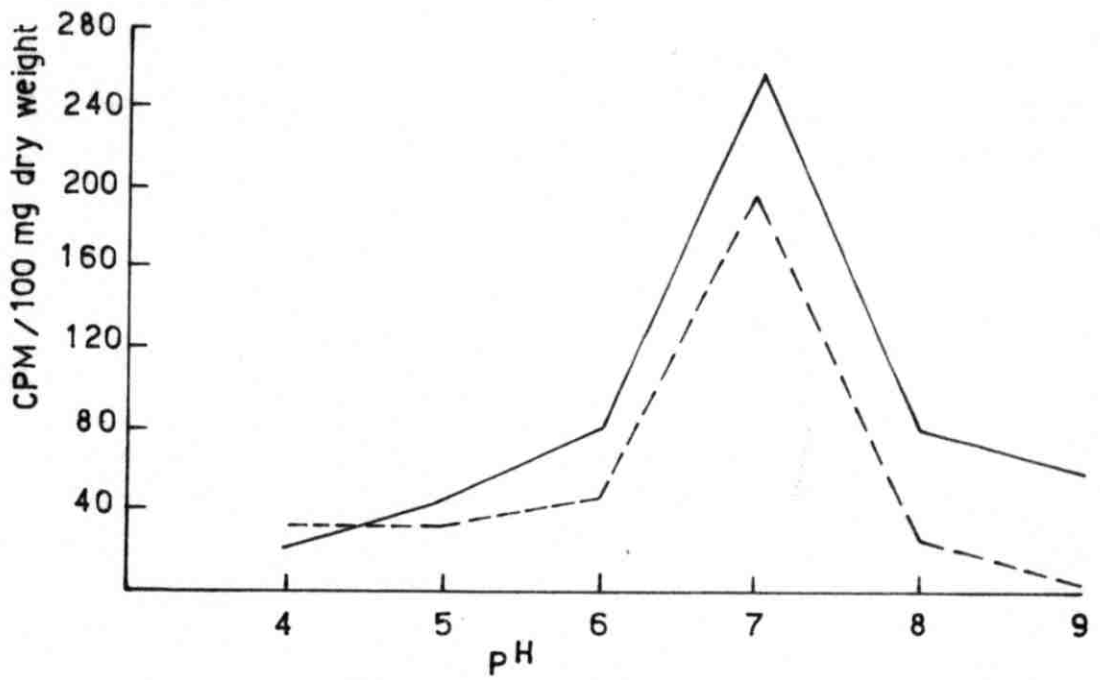

Figure 9. Depletion of Zn^{65} from the external nutrient solution in 24 and 48 hrs

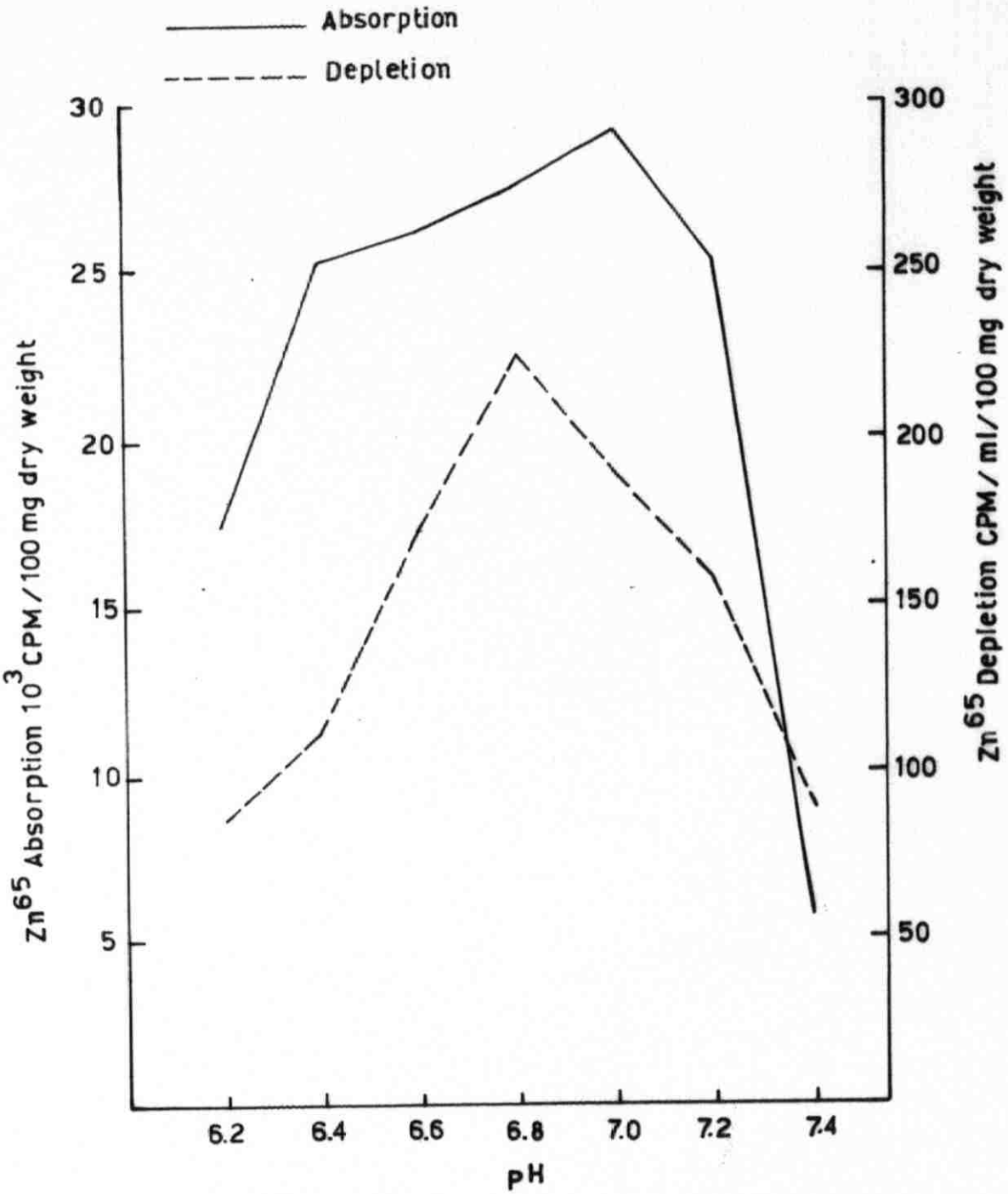

Figure 10. Average Zn^{65} absorption by the whole plant in 24 hrs., and average depletion in the external solution at various pH values

Table 8

Effect of pH on the absorption and translocation of Zn^{65} in 24 and 48 hours.

pH	leaves cpm/100 mg			stem cpm/100 mg			root cpm/100 mg			whole plant cpm/100 mg			depletion	
	24 hrs	48 hrs	factor	24 hrs	48 hrs	factor	24 hrs	48 hrs	factor	24 hrs	48 hrs	factor	24 hrs	48 hrs
4	46	677	14.7	1042	3233	3.1	23503	36777	1.6	8197	13563	1.7	33	26
5	57	557	9.8	1094	3360	3.1	26532	47868	1.8	9228	17262	1.9	35	51
6	104	474	4.6	1690	3240	2.0	46524	58643	1.3	16106	20786	1.3	46	81
7	117	326	2.8	1939	3174	1.6	60884	117436	1.9	20980	40312	2.0	202	279
8	37	59	1.6	394	455	1.2	9681	11964	1.2	3371	4159	1.2	29	100
9	29	52	1.8	222	487	2.2	6271	15295	2.4	2175	5278	2.1	10	62

Table 9

The effect of various pH values on the absorption and translocation of Zn^{65} in pinto bean seedlings in 24 hours.

pH	cpm / 100 mg dry weight					
	leaves	stem	root	whole plant	deplation	pH change
6.2	15	1271	50958	17415	88	-.16
6.4	76	1907	76542	26175	106.5	-.19
6.6	34	1071	78847	26651	174.5	-.13
6.8	95	1325	80335	27245	229	-.15
7.0	46	1446	87635	29709	181	-.05
7.2	30	1026	81592	27549	165	-.23
7.4	56	458	19876	6797	97	-.56

below pH 6.2 and above 7.2.

Translocation of Zn^{65} to the stem and the primary leaves was favored by acidic solution. Very low translocation to the shoot occurred in plants grown in the alkaline solutions.

The least pH change in the nutrient solution within 24 hours, was noticed at pH 7.0. The decrease was about 0.05 as compared to a decrease of 0.56 at pH 7.4.

Because of the above findings, pH 6.8 was considered the optimum pH for the highest Zn^{65} absorption and translocation. The pH of all the nutrient solutions used for the study of other variables in the present investigation was adjusted to 6.8.

Soil experiment.

The results obtained in the nutrient solution experiments were tested in the soil experiment. The four soil samples used were collected at random from different regions of Lebanon at various altitudes, as shown in Table 10 and Plate 1. The soil samples were different is texture and may be in composition and other characters, but all were similar in having alkaline pH. A fifth sample was prepared by just adjusting the pH of one sample to 7.0, which makes comparison meaningful and rules out the effects of other factors than the pH value. The results, as shown in Figure 11 and Table 10, indicate that Zn^{65} absorption decreased as the pH increased in the alkalineside of the scale.

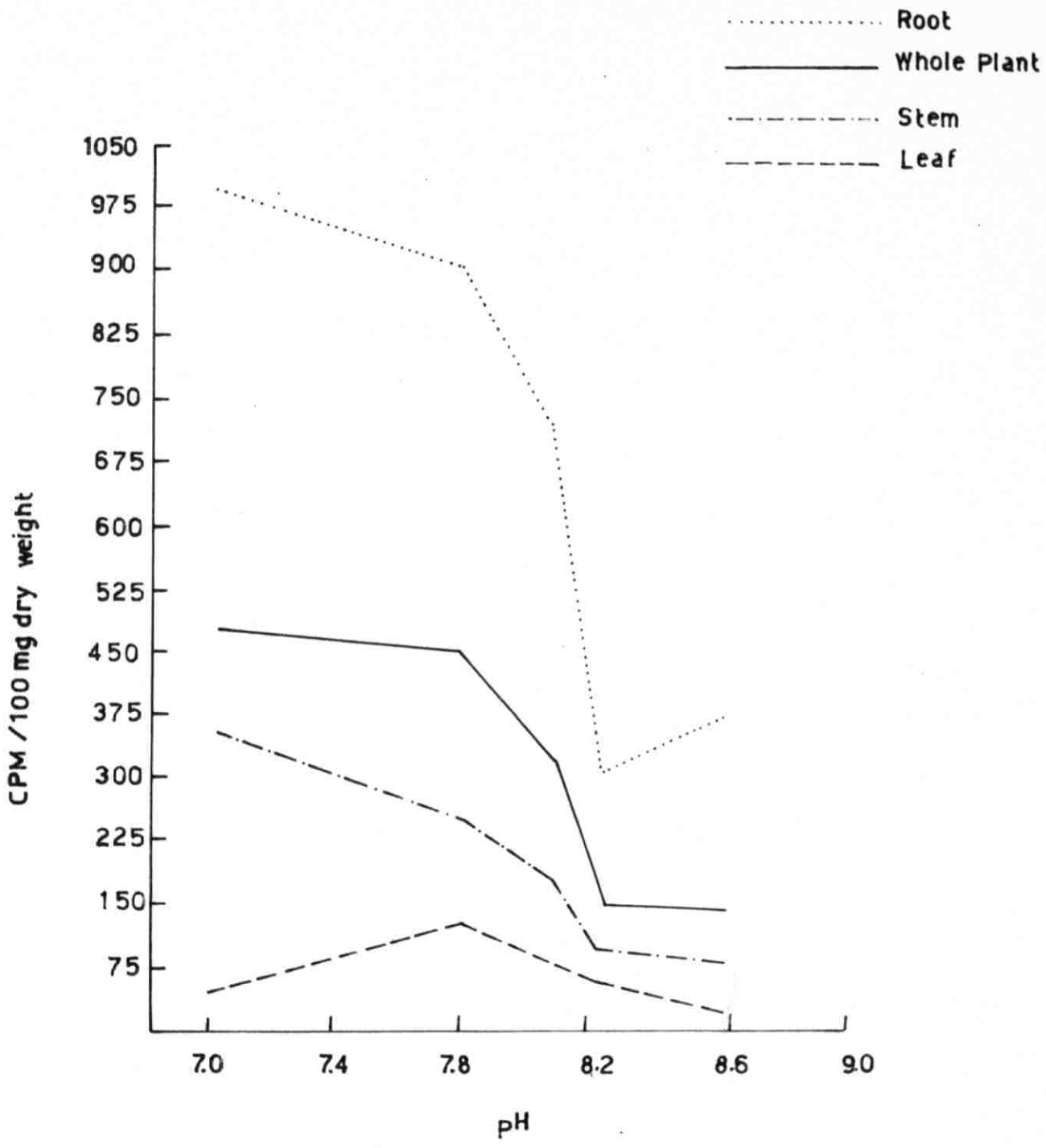

Figure 11. Effect of Soil pH on the absorption and translocation of Zn^{65}

Table 10

"Average absorption and translocation of Zn^{65} from soils having different pH values"

Soil origin	Altitude (meters)	Soil structure	PH	cpm/100 mg dry weight						
				leaves	stem	root	whole plant	root/ leaves	stem/ leaves	% translocated to shoot
Beirut[1]	30	sandy	7.00	37	350	1028	472	28	10	27.3
Jbaa	1000	clay	7.80	92	284	902	426	10	3	29.4
Jdaideh	800	sandy clay loam	8.10	66	175	741	327	11.2	2.7	24.5
Tripoli	125	heavy clay	8.25	28	105	314	149	11.2	3.8	29.7
Beirut	30	sandy	8.65	21	82	341	148	4.2	4.0	23.2

1. Soil pH was adjusted to 7.0 by the addition of an appropriate amount of 0.01 N H_2SO_4.

The interference of other soil factors can be excluded only by comparing the plants of group 1 with the plants of group 5. All the conditions, under which both groups were grown, were the same, especially the soil texture and composition, except the pH value which was adjusted to pH 7.0 in the case of group 1.

The amount of Zn^{65} absorbed by the whole plants of group 1 was three times as much as the amount absorbed by group 5. The amount of zinc translocated to the stems of group 1 was more than four times greater than translocated zinc in group 5. The leaves showed fluctuation of Zn^{65} content, but the least amount existed in the primary leaves of the plants grown in soils having the highest pH values, namely 8.25 and 8.65.

Thus, the natural soil experiment confirms the results obtained in the nutrient solution, at least at pH levels above 7.0.

Effect of light.

Three lighting conditions were studied in this experiment. Table 11 shows the results obtained. It was noticed that the continuous illumination for 24 hours with light intensity as high as 3000 lumens did not cause the highest zinc absorption. More zinc was absorbed by the roots of the plants which were grown in complete darkness. Translocation of Zn^{65} to the shoot was higher

in plants exposed to light. Continuous illumination resulted in a higher content of Zn^{65} in the stem. 16.7% of translocated Zn^{65} was taken up by the leaves of plants grown under "natural" lighting conditions, day-light and night-darkness; while 8.7% of translocated zinc was taken up by the leaves of plants grown under continuous illumination of high light intensity. These results support the observations of Viets (1966), who stated that the sunny side of trees showed more zinc deficiency than the shady side.

The pinto bean plants provide an excellent experimental material, since one of the primary leaves can be used as a control for the experimental opposite leaf. This was done in studying the effect of light on Zn^{65} accumulation in primary leaves.

As it is shown in Table 12, the exposed primary leaf of the plant accumulated within 48 hours 7 times as much Zn^{65} as the covered leaf. This, probably, was due to the higher metabolic activities in the exposed leaf. The two opposite primary leaves of those plants which were kept in complete darkness for 48 hours accumulated less than half the amount of Zn^{65} accumulated by the single exposed leaf.

As to the zinc content of the roots, the results obtained in this experiment contradict those obtained in the previous experiment. This deviation may be due to the different durations of exposure used in the two experiments.

Table II

"Effect of light on the absorption and translocation of Zn^{65} in pinto bean seedlings"

Illumination	cpm / 100 mg dry weight				Depletion	percent translocation	
	leaves	stem	root	whole plant		leaves	stem
Continuous illumination	155	1628	42345	14709	92	8.7	91.3
Day-Night	289	1439	40674	14134	103	16.7	83.3
Complete darkness	82	1286	43606	14991	97	6.0	94.0

Table 12

Absorption and translocation of Zn^{65} in 48 hours under different lighting conditions

Treatment	cpm / 100 mg dry weight				depletion
	covered leaf	exposed leaf	stem	root	
1 leaf covered	67	477	4809	77145	89.6
whole plant in the dark	202	--	4439	70804	67.0

The diurnal variation.

The results of the experiment designed to study the diurnal variation in Zn^{65} absorption and translocation are presented in Figures 12 and 13 and Table 13.

The maximum amount of Zn^{65} taken up by the whole plant was during the period from 2 - 6 A.M.. This was a direct result of the root effect because the highest root absorption took place during the same period. This was immediately followed by the period of the next highest absorption by the root, 6 - 10 A. M, which was the period of the maximum accumulation in the leaves.

The maximum translocation of Zn^{65} to the stem, took place during day hours, especially between 6 A.M. - 2 P.M. In the case of Zn^{65} accumulation in the leaves, there were apparently two factors resulting in the two high levels noticed.The highest Zn^{65} accumulation in the leaves occurred between 6 - 10 A.M., immediately after the period of maximum root absorption, suggesting a root effect. The second highest Zn^{65} accumulation in the leaves was between 10 A.M. - 2 P.M. during midday, probably due to an increase in metabolic activities.

Effect of temperature.

Table 14 shows the influence of temperature on Zn^{65} absorption and translocation. Lowering the temperature from 25°C.

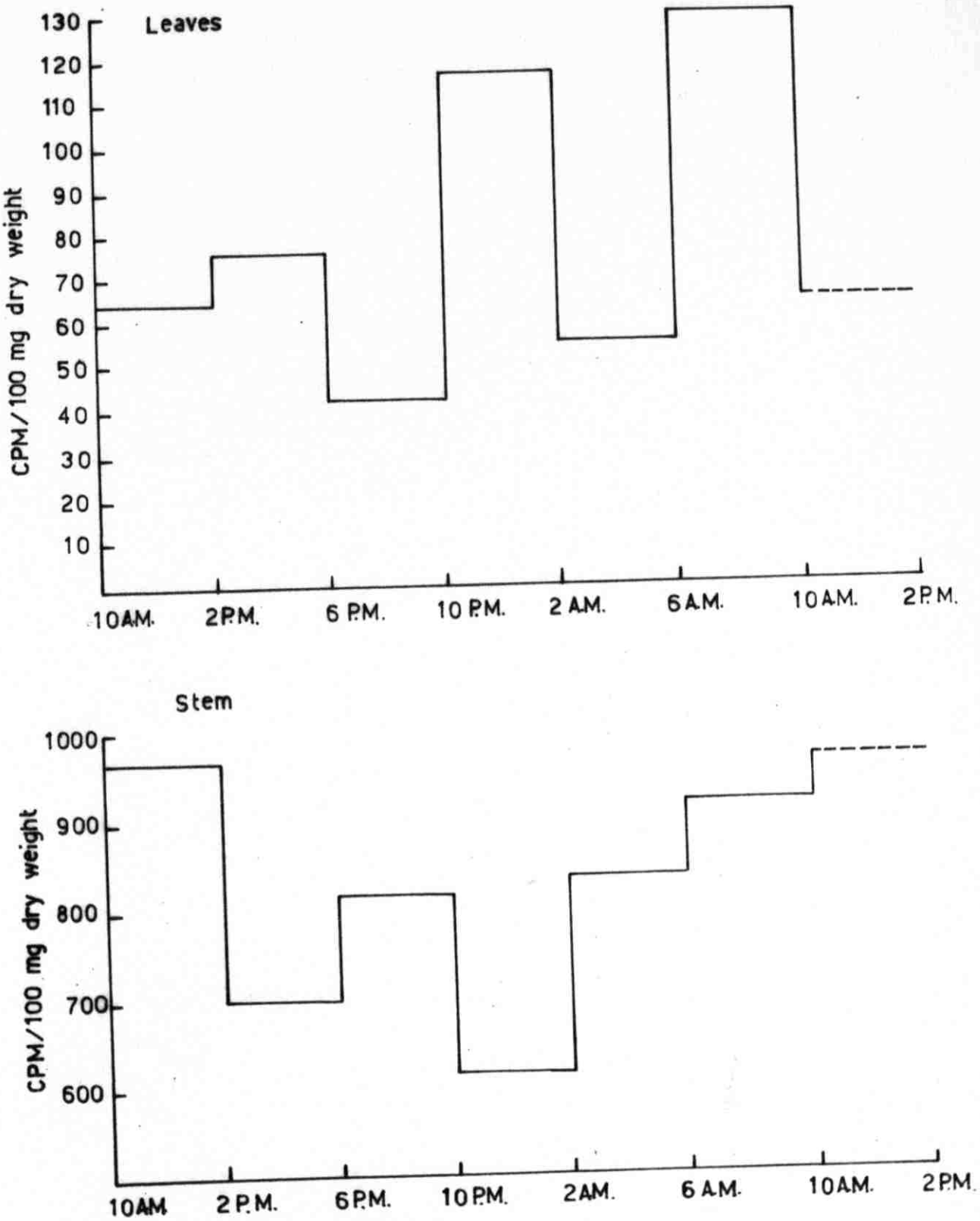

Figure 12. The diurnal variation in the absorption and translocation of Zn^{65} in Pinto bean seedlings.

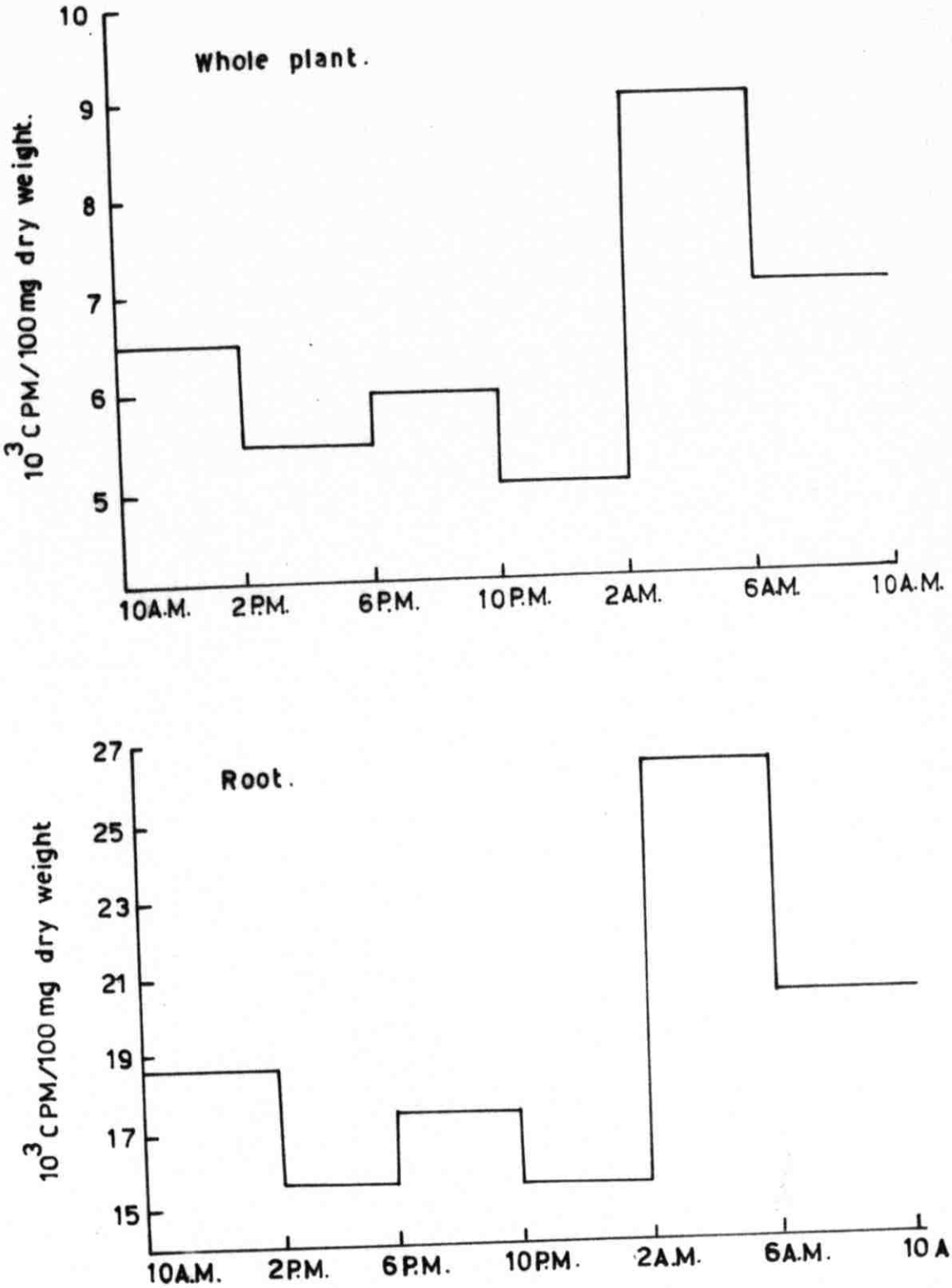

Figure 13. The diurnal variation in the absorption and translocation of Zn^{65} in Pinto bean seedlings.

Table 13

The diurnal variation in the absorption and translocation of Zn^{65} in Pinto bean seedlings.

Duration	cpm/100 mg dry weight				Depletion cpm/1 ml/100 mg dry weight
	leaves	stem	root	whole plant	
10 am - 2pm	67	959	18483	6503	65
2 pm - 6pm	73	700	15453	5409	75
6 pm - 10pm	43	818	17452	6102	85
10 pm - 2pm	118	602	15528	5176	39
2 pm - 6pm	55	886	26715	9219	78
6 am - 10pm	120	917	20260	7099	71

Table 14

"Effect of temperature on the absorption and translocation of Zn^{65} in Pinto bean seedling "

Temperature	cpm / 100 mg dry weight				depletion	Percent translocated to shoot
	leaves	stem	root	whole plant		
$25^{\circ} \pm 2$ C	118	2026	34521	12222	163	6 %
15° C	83	1272	35714	12356	144	3.7 %
5° C	57	797	24109	8221	79	3.4 %

to 15°C. caused no or little change in Zn^{65} absorption. The results obtained revealed, unexpectedly, a slight increase in root absorption when the temperature was lowered to 15°C. On the other hand, a decrease in the temperature from 25° to 15 and 5°C. caused a decrease in Zn^{65} translocated to the shoot. The higher temperatures favored zinc transfer to the shoot.

The Q_{10} obtained between 5° and 15°C. was 1.5 which is quite reasonable for cation absorption. The depletion of zinc from the external solution at 15°C was twice as much depletion occurred at 5°C.

Effect of age.

In oat plants, it was found by William and Moore(1952) that the zinc content of the whole plant decreased from an initially high value until flowering stage after which there were little further changes. This does not seem to be true in the case of Pinto bean plants. As shown in Talbe 15 and Figures 14 and 15, younger plants are less efficient in zinc absorption. This may be due to the fact that more developed and larger roots can absorb and transfer more zinc.

The young immature leaves received more zinc than the older ones. This was clear both in primary and compound leaves.

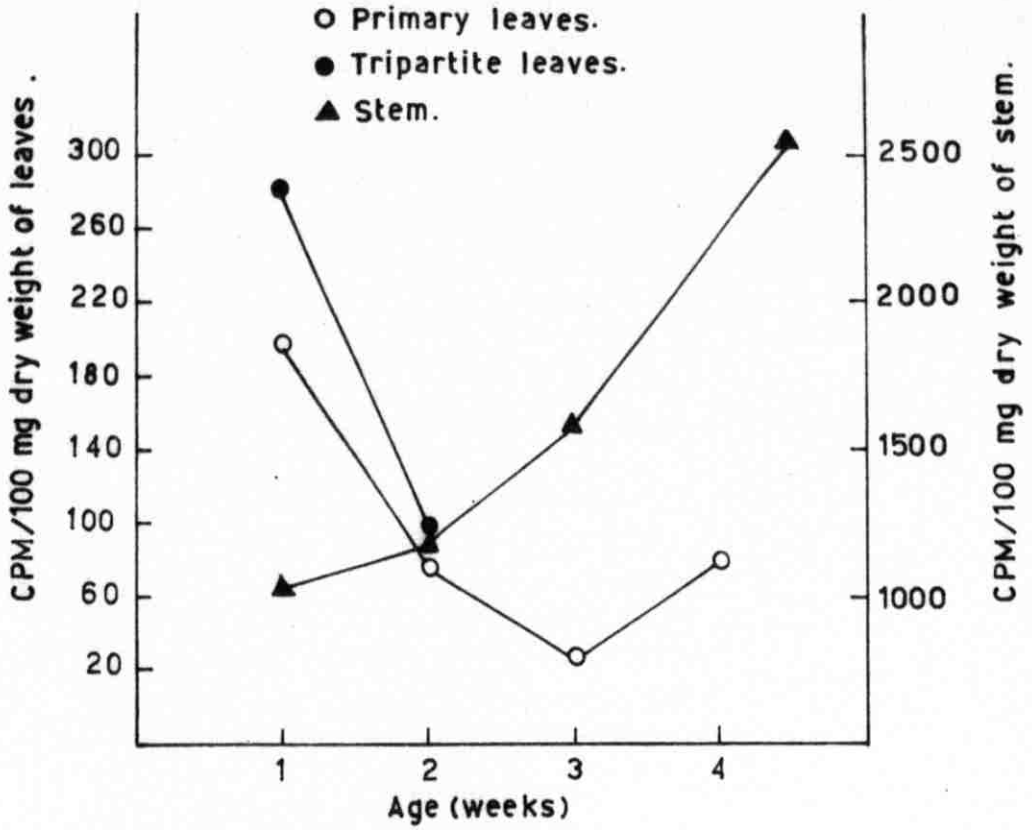

Figure 14. Effect of age on the translocation of Zn^{65}.

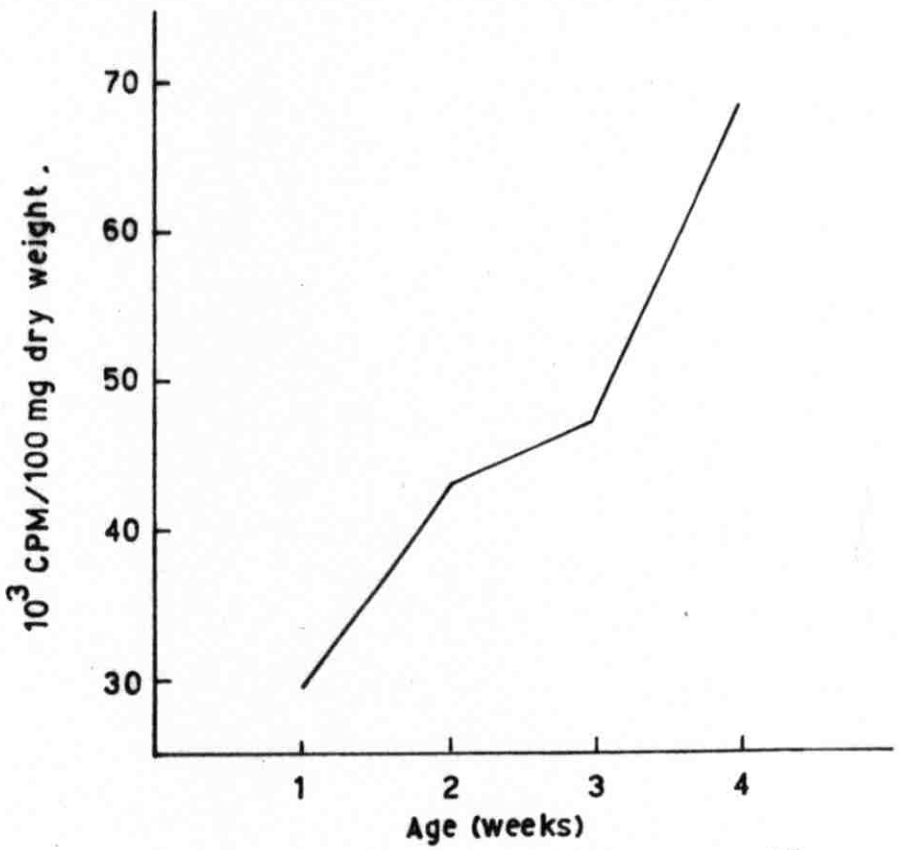

Figure 15. Effect of age on the absorption of Zn^{65}.

Table 15

Effect of age on Zn^{65} absorption and translocation in Pinto bean plants.

Age (weeks)	cpm / 100 mg dry weight						
	Primary leaves	comp. leaves	stem	root	whole plant	depletion	Percent translocated to shoot
1	197	--	1036	29948	10394	214	4.0
2	73	--	1228	43724	15008	174	3.0
3	22	281	1589	46802	12174	188	3.9
4	79	98	2551	67054	17645	152	3.9

Zn^{65} content was higher in parts of the plant that had active growth and consequently active metabolic processes. The one-week old primary leaves contained about three times the amount of Zn^{65} in the two-week old primary leaves. The one-week old compound leaves contained also about three times as much Zn^{65} as in the two-week old compound leaves. The stem content of Zn^{65} was directly proportional to the age of the stem, and the amount of Zn^{65} available for translocation from the root.

Translocation of Zn^{65}.

In addition to the afore mentioned effects of various factors on the translocation of Zn^{65} in Pinto bean seedlings, a few experiments were carried out to study the distribution and transfer of the radioactive zinc within the intact plant.

Table 16 shows the results of one experiment in which the plants were transferred from the radioactive solution to nutrient solutions containing no Zn^{65} for various intervals. As it is indicated in Table 16, with time there was a continuous decrease in Zn^{65} content of the root. The stem and leaves did not show any gain of new amounts of Zn^{65}. In fact there was a decrease also in the initial Zn^{65} content of the stem and the leaves, probably due to cation exchange over a long period of time (up to 72 hours).

Table 16

Effect of post treatment with nutrient solution on the loss and translocation of Zn^{65}.

Duration of post treatment with nutrient solution	cpm/100 mg dry weight					
	leaves	stem	root	root/ stem	stem/ leaves	root/ leaves
0	165	2027	74202	37	12	450
24	80	1515	40879	27	19	511
48	65	1499	34225	23	23	527
72	123	1560	33518	22	12.7	27.3

Plate 2 shows the autoradiogram of a plant grown in a nutrient solution containing Zn^{65} for 7 days. The highest accumulation of Zn^{65} was noticed in the root, and less amounts were translocated to the shoot. Along the stem a higher concentration of $zinc^{65}$ was observed at the bases of the primary and tripartite leaves.

Results of foliar application are presented in Tables 17 and 18. When $Zn^{65}Cl_2$ was applied to the upper and lower surface of the plant leaves, it was noticed that more Zn^{65} was absorbed by the lower surface. A primary leaf, the lower surface of which was brushed by $Zn^{65}Cl_2$, retained 23392 cpm/100 mg dry weight, while 14509 cpm/100 mg were retained by a leaf when $Zn^{65}Cl_2$ was applied to the upper surface. The opposite primary leaves of both plants received about the same amount of Zn^{65}. Translocated Zn^{65} to the stem and root was much higher in the plant which received lower surface treatment.

When drops of $Zn^{65}Cl_2$ were placed on different parts of the upper surface of the blade, it was found, as indicated in Table 18, that absorption from the basalregion was higher than absorption from the tip region. Translocation of Zn^{65} to other parts of the plant was higher when the element was applied to the tip of the leaf.

Translocation of Zn^{65} injected into different parts of the plant is represented in Plates 3, 4 and 5.

Table 17

Foliar application of Zn^{65} to the upper and lower surface of plant leaves.

Plant part	Upper surface treated		Lower surface treated	
	Weight mg.	cpm/100 mg. dry weight	Weight mg.	cpm/100 mg. dry weight
Treated leaf	35	14509	44.5	23392
Opposite leaf	35.5	258	41.5	243
Stem	98	53	110.5	96
Root	30	13	37	596
Percent translocated		2.2		4.0

Table 18

Foliar application of Zn^{65} to different regions of the upper surface of the leaves.

Plant part	cpm/100 mg dry weight	
	Tip application	Base application
Treated leaf	22192	34820
Opposite leaf	707	277
Stem	48	47
Root	854	452

The highest translocation was obtained when Zn^{65} was injected into the stem near the scars of the cotyledons. When the injection was made into the base of the stem, translocation to the root and up through some length of the stem was noticed.

When Zn^{65} was injected into the petiole of one primary leaf, no translocation to the opposite leaf was noticed in 24 hours. More Zn^{65} was translocated to the stem and the root.

V. SUMMARY AND CONCLUSIONS.

1. The study of factors influencing absorption and translocation of Zn^{65} in Pinto bean seedlings grown in nutrient solution revealed the following: The presence of cations other than Zn^{++}, or the low pH of the solution decreased Zn^{65} absorption. The factors which caused a decrease in Zn^{65} absorption by the root, favored translocation of Zn^{65} to the shoot.

2. Treatment of the roots of intact Pinto bean seedlings with 0.01 M $CaCl_2$ for a period ranging from 0 to 60 minutes after Zn^{65} absorption experiments, showed no significant decrease in the Zn^{65} content of the root. It caused a marked decrease in the translocated Zn^{65}.

3. Pinto bean seedlings showed a continuous increase in the rate of Zn^{65} absorption up to 128 minutes. "Saturation" of the root with Zn^{65} was not reached in a period of 128 minutes.

4. An increase in the concentration of the stable isotope up to 50 ppm in the nutrient solution caused a decrease in Zn^{65} absorption by Pinto bean seedlings. The Zn^{65} absorption was inversely proportional to the concentration of stable zinc in the nutrient solution.

5. An increase in the concentration of Zn^{65} up to 14 ucu/liter, both in distilled water and in complete nutrient solution, caused an increase in the absorption and translocation of the element within the plant.

6. Previous treatment of Pinto bean plants with stable zinc for one week showed that the highest absorption of Zn^{65} occurred when the plants were previously grown in a nutrient solution containig 2.5 ppm of stable zinc.

7. The maximum absorption of Zn^{65} occurred when Pinto bean seedlings were grown in nutrient solution having a pH range from 6.2 to 7.2. The highest root content was found at pH 7.0. Maximum translocation to the stem and the leaves was at acidic pH values.

8. The four soil samples collected at random from different areas of Lebanon had a pH range from 7.8 to 8.65. Pinto bean seedlings absorbed less Zn^{65} from soils having high pH values. Translocation of Zn^{65} to the shoot was higher in Pinto bean plants grown in soils having low pH values.

9. The highest accumulation of Zn^{65} in the roots was found to occur in Pinto bean plants grown in complete darkness for 24 hours. Natural lighting conditions (alternating day and night) caused the highest translocation of Zn^{65} to the leaves.

10. Determination of Zn^{65} accumulation in light and complete darkness in fully comparable opposite leaves of several plants, showed that on the average the exposed leaves had about twice the amount of that kept for the same time in the dark. Primary leaves exposed to light accumulated in 48 hours 7 times as much Zn^{65} as accumulated by the covered opposite leaves on the same plants.

11. Diurnal variation was found to occur in Pinto bean seedlings as to absorption and translocation of Zn^{65} as follows: The highest root absorption occurred at 2 A.M. to 6 A.M., while the highest translocation to the stem took place from 6 A.M. to 2 P.M. Translocation to the leaves was best during the day hours.

12. Raising or lowering the temperature from 15° to 25°C. or from 25° to 15°C. caused no or little change in Zn^{65} absorption by Pinto bean seedlings. Raising the temperature from 5° to 15° C. caused an increase in Zn^{65} absorption. Lowering the temperature from 15° to 5° C. caused a decrease in Zn^{65} absorption. The higher temperatures (25° and 15°C.) favored Zn^{65} translocation to the shoot. The Q_{10} (from 5° to 15°C.) obtained was 1.5.

13. The highest absorption and accumulation of Zn^{65} was found to occur in the roots and stems of four-week old Pinto bean plants. Younger leaves, primary and compound, accumulated more Zn^{65} than older ones.

14. The autoradiograms showed that the root always contained a higher concentration of Zn^{65} than the shoot. Along the stem, more Zn^{65} accumulated in the nodes or at the bases of leaves.

15. More Zn^{65} was absorbed by the lower surface of the leaf than the upper suface.

16. More Zn^{65} was absorbed when applied to the basal region of the upper surface of the leaf than the apex. But, more Zn^{65} was translocated to the stem and the root when applied to the apical region of the upper surface than the basal region.

VI. LITERATURE CITED.

Adelstein, S.J. and Vallee, B.L. 1956. In P.D.Boyer et al.(eds.). The Enzymes III., Academic Press, New York.

Alben, A.O. and Boggs, H.M. 1936. Zinc content of soils in relation to pecan rosette. Soil Sci. 41:329

Allard, R.W. and Smith L. 1954. Dry edible bean production in California. (Calif. Agr. Exp. Sta. Circular 436)

Allison, R.V., Bryan, O.C. and Hunter, J.H. 1927. The stimulation of plant response on the raw soil of the Florida Everglades through the use of Copper and other chemicals. Florida Agr. Expt. Sta. Bull. 190:33 - 80

Altenbern, R.A. and Housewright, 1953 R.D. In Dixon, M. and Webb, E.C. Enzymes, Longmans, Green and Co., London.

Apple, S.B. and Butts J. S. 1953. Soil temperature studies : 1- The effect of soil temperature and phosphorus on growth and phosphorus uptake by Pole beans. Proc. Amer. Soc. Hort. Sci. 61:325-332.

Arnon, M. L. In P. D. Boyer et al.(eds.).The Enzymes III,. Academic Press, New York.

Arnon, D. I. and Hoagland D. R. 1940. Crop production in artificial solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil. Sci. 50:463-484

Baily, K. and Webb, E. C. 1948. In Dixon, M. and Webb, E. C. Enzymes, Longmans, Green and Co., London.

Bauer, A. and Lindsay, W. L. 1965. The effect of soil temperature on the availability of indigenous soil zinc. Soil Sci. Soc. Amer. 29:413-416

Barnette, R. M., Camp, J. P. and Warner, J. D. 1935. Study of chorosis in corn plants and other field crop plants. Florida Agr. Exp. Sta. Ann. Rpt., 1943. 49+50

Beisenherz, G. et al. In P. D. Boyer et al. (eds.). The Emzymes III., Academic Press, New York.

Bertrand, G. and Vladesco, R. 1920. Distribution of zinc in the horse. Compt. Rend. Acad. Sci. Paris 171:744-746

Bhatia, I. S. et al. 1955. In Dixon M. and Webb, E. C. Enzymes, Longmans, Green and Co., London.

Bliss, A. F. 1949. In Boyer P.D. et al.(eds.) The Enzymes III, Academic Press, New York.

Bliss, A. F. 1951. In Boyer P. D. et al. (eds.) The Enzymes III, Academic Press, New York.

Biddulph, O., Cory, R. and Biddulph, S. 1959. Translocation of calcium in the bean plant. Pl. Physiol. 34:512-519

Bidwell, E. and Van Heyningen W. E. 1948. In Dixon, M. and Webb, E. C. Enzymes. Longmans, Green and Co., London.

Boawn, L. C. and Leggett, G. E. 1964. Phosphorus and zinc concentrations in Russet Burbank Potato tissues in relation to development of zinc-deficiency symptoms. Soil. Sci. Soc. Amer. 28:229-232.

Boawn, L., Viets, F. and Crawford, C. 1954. Effect of phosphate fertilizers on zinc metabolism of field beans. Soil Sci. 78:1-7

Bodansky, M. 1920. Biochemical studies on marine organisms: II The occurrence of zinc. Jour. Biol. Chem. 44:399-407

Bonner, J. and Varner, J. E. (eds.) Plant Biochemistry, Academic Press, New York.

Bonnichsen, K. 1950. In Colowick, S. P. and Kaplan, N. O. (eds.), 1955. Methods in Enzymology, Academic Press, New York.

Bonnichsen, R. K. and Wassen, A. M. 1948. In Boyer, P. D. et al. (eds.) The Enzymes III, Academic Press, New York.

Boursnell, J. C. 1958. Safety Techniques for Radioactive Tracers. Cambridge University Press, England.

Bowness, J. M., Morton, R. A., and Shakir, M. H. and Stubbs, A. L. 1952. In Lamb, C. A. et al. (eds.). Trace Elements. Academic Press,New York.

Boyer, P. D., Lardy, H. and Myrback, K. 1959. The Enzymes,I, Academic Press, New York.

Boyer, P. D., Lardy, H. and Myrback, K. 1960. The Enzymes III. Academic Press, New York.

Brenchley, W. E. 1914. On the action of certain compounds of zinc, arsenic and boron on the growth of plants. Ann. Bot., Lond 28:283

Brinkman, R., Margaria, R., Meldrum, N. V. and Roughton, F. T. 1932. In Boyer, P. D. et al.(eds.) The Enzymes III. Academic Press, New York.

Brownhill, T., Jones, A. and Stacey, M. 1959. The inactivation of ribonuclease during the isolation of ribonucleic acids. Biochem. J. 73:434-438

Broyer, T. C. Further observations on the absorption and translocation of inorganic solution using radioactive isotopes with plants. Pl.Phys. 25:367-376

Brusca, J. N. and Haas, A. R. 1959. zinc effect on citrus and avocado. Calf. Agric. 13, No. 1, 12

Burleson, C. A., Dacus, A. D. and Gerard, C. J. 1961. The effect of phosphorus fertilization on the zinc nutrition of several irrigated crops. Soil Sci. Soc.Amer.25:365-368

Callender, G. R. and Gentzkow, C. J. 1937. Acute poisoning by the zinc and antimony content of limeade prepared in galvanized iron can. Mil. Surg. 80:67-70

Camp, A. F. 1945. Zinc as a nutrient in plant growth. Soil Sci. 60:157-164.

Chandler, W. H. 1937. Zinc as a nutrient for plants. Bot. Gas. 98:625-646.

Chandler, W. H., Hoagland, D. R. and Hibbard, P. L. 1931. Little leaf or rosette in fruit trees I. Proc. Amer. Soc. Hort. Sci. 28:556-560

Chandler, W. H., Hoagland, D. R. and Hibbard, P. L. 1934. Little leaf or rosette of fruit trees IV. Proc. Amer. Soc. Hort. Sci. 31:11-19.

Chesters, C. G. and Rolinson, C. 1951. Trace elements and streptomycin production? J. Gen. Microbiol. 5:559-565

Chesters, C. G. and Rolinson, C. 1951. Zinc in the metabolism of Aspergillus niger. J. Gem. Microbiol 5:553-558

Churbanov, V. M. 1959. Results of investigations on the uptake, movement and distribution of trace elements in plants using tracer atoms. Primen. Mikroelem. Sel. Khoz. Baku 1958, 139-143

Colowick, S. P. and Kaplan, N. O. 1955. Methods in Enzymology II, Academic Press, New York.

Comar, C. L. 1955. Radioisotopes in Biology and Agriculture. McGraw-Hill Book C. Inc., New York.

Crafts, A. S. 1967. Bidirectional movement of labelled tracers in soybean seedlings. Hilgardia 37:No.16:625-638

Davidson, D. C. 1951. In Boyer, P. D. et al. (eds.) The Enzymes III. Academic Press, New York.

Day, B. E. 1952. The absorption and translocation of 2,4-D by bean plants. Plant Phys. 27: 143-154

Day, H. G. 1942. Effects of zinc deficiency in the mouse. Fed. Proc. 1:188

Day, H. G. and McCollum, E. V. 1940. Effects of acute dietary zinc deficiency in the rat. Proc. Soc. Expt. Biol. and Med. 45:282-292

Day, H. G. and Skidmore, B. E. 1947. Some effects of dietary zinc deficiency in the mouse. Jour. Nutr. 33:27-38

Day, R. and Franklin, J. 1946. Plant carbonic anhydrase contains zinc. Science 104:363

Dixon, M. and Webb, E. 1958. Enzymes. Longmans, Green and Co., London, England.

Early, E. B. 1943. In Hewitt, E. J. Sand and water culture Methods

used in the study of plant nutrition. Commonwealth Agricultural Bureaux. Farnham, Bucks, England.

Eaton, F. M. 1941, In Hewitt, E. J. Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureaux. Farnham, Bucks, England.

Ellis Jr., R., Davis, J. F. and Thurlow, D. L. 1964. Zinc availability in calcarious Michigan soils as influenced by phosphorus level and temperature. Soil Sci. Soc. Amer. 28:83-86

Elvehjem, C. A. and Hart, E. B. 1934. Lack of zinc prevents normal hair growth in rats. Wisconsin Agr. Expt. Sta. Bull 428:23-24

Epstein, E. and Stout, P. R. 1961. The micronutrient cations iron, manganese, zinc and copper: Their uptake by plants from the absorbed state. Soil Sci. 72:47-64

Floyd, B. F. 1917. Some cases of injury to citrus trees apparently induced by ground limestone. Florida Agr. Exp. Sta. Bull. 137:161-179

Folk, J. E., Piez, K. A. Carrol, W. R. and Gladner, J. A. 1960. Carboxypeptidase B. IV. Purification and characterization of the porcine enzyme. J. Biol. Chem., 235:2272

Foster, J. N. and Denison, F. W. 1940. In Lamb, C. A. et al.(eds.) Trace Elements. Academic Press, New York.

Francis, G. E., Mulligan, W. and Wormall, A. 1959. Isotopic Tracers. 2nd ed. The Athlone Press, University of London.

Fuehring, H. D. and Soofi, G. S. 1964. Nutrition of corn on a calcareous soil: II. Effect of zinc on the yields of grain and stover in relation to other micronutrients. Soil. Sci. Soc. Amer. 28:79-82

Ghigliotto, C. 1919. The normal existence of zinc in the human organism. Ann. Falsif 12 No. 123, 124 pp12-14.

Giaya, S. 1920. Zinc in the human organism. Compt. Rend. Acad. Sci. Paris, 170:906-909

Gilbert, F. A. 1957. Mineral Nutrition and the Balance of Life. Norman: University of Oklahoma Press, U. S. A.

Gregolin, C. and Singer, T. P. 1963. The lactic dehydrogenase of yeast, III:D(-)lactate cytochrome C reductase, a zinc flavoprotein from aerobic yeast. Biochim. Acta, 62:201

Guest, P. L. and Chapman, H. D. 1944. In Hewitt, E. J. Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureaux. Farnham, Bucks, England.

Haas, A. R. 1932. In Hewitt, E. J. Sand and water culture methods used in the study of plant nutricition. Commonwealth Agricultural Bureaux. Farnham, Bucks, England.

Hallsworth, F. G. 1958, Nutrition of the legumes. Butterworths Scientific Publications, London.

Hanson. J. B. and Biddulph, O. 1953. The diurnal variation in the translocation of minerals across bean roots. Plant Phys., 28:356-370

Harrison, J. H. 1963. Participation of Zn^{+2} in mechanism of malic dehydrogenase. Fed. Proc., 22:493

Hegsted, D., McKibbin, J. M. and Drinker, C. K. 1945. Biological, biogenetic and medical properties of zinc and zinc compounds. U. S. Pub. Health Repts. Suppl. 179:44 pp.

Hevesy, G. 1948. Radioactive Indicators, Interscience Publishers Inc., New York.

Hewitt, E. J. 1966. Sand and water culture Methods Used in the Study of Plant Nutrition, Commonwealth Agricultural Bureaux, Farnham, Bucks, England.

Hibbard, P. L., 1940. Asoil zinc survey in California. Soil Sci. 49:63-72

Hiltner, R. S. and Wichmann, H. J. 1919. Zinc in Oysters. Jour. Biol. Chem. 38:205-221

Hoagland, D. R., Chandler, W. H. and Stout, P. R. 1936. In Truog, E. (ed.) Mineral Nutrition of Plants. The University of Wisconsin Press.

Hoch, F. L. and Vallee, B. L. 1949. In Lamb, C. A. et al.(eds.) 1958. Trace Elements. Academic Press, New York.

Hoch, F. L. and Vallee, B. L. 1958. In Lamb, C. A. et al.(eds.) 1958. Trace Elements. Academic Press, New York.

Holmberg, C. G. 1939. Uricase purification and properties. Biochem. J. 33:1901-1906

Holmes, R. S. 1943. Copper and zinc contents of certain United States Soils. Soil Sci. 56:359-370

Holzer, H. and Schneider, S. 1955. In Boyer, P. D. et al.(eds.) The Enzymes, III. Academic Press, New York.

Hove, E., Elvehjem, C. A. and hart, E. B. 1938. Further studies on zinc deficiency in rats. Amer. Jour. Physiol. 124:750-758

Husz, B. 1940. In International Union of Biological Science, Trace Elements in Plant Physiology., Chronica Botanica Company, Waltham, Mass. U. S. A.

Jagannathan, V., Singh, K., and Damodara, M. 1956. Carbohydrate Metabolism in citric acid fermentation. IV Purification and properties of aldolase from Aspergillus niger. Biochem. J. 63:94

Javillier, M. 1908. On the occurrence of zinc in plants. Ann. Inst. Pasteur 22:720-727

Jurinak, J. and Thorne, D. 1955. Zinc solubility under alkaline conditions in a zinc-bentonite system. Soil Sci. Soc. Amer. Proc. 19:446-448

Kamen, M. D. 1951. Radioactive Tracers In Biology, 2nd ed. Academic Press, New York.

Keilin, D. and Mann, T. 1940. Carbonic anhydrase, purification and nature of the enzyme. Biochem. J. 34:1163-1176

Kickli, E., and Edsall, J., 1961. Zinc binding and the sulfhydryl group of human carbonic anhydrase. J. Biol. Chem., 237: Pc 258

Kornberg, A. and Pricer, W. 1951. In Boyer, P. D. et al.(eds.) The Enzymes, III. Academic Press, New York.

Kuwabara, S. and Abraham, E. P. 1967. Some properties of two extracellular B-lactamases from Bacillus cereus 5691H. Biochem. J. 103:27c-30c

Labanauskas, C. K., Embleton, T. W. and Jones, W. W. 1958. Deficiencies of micronutrients. Cal. Agric. 12: No 10,10

Lamb, C. A., Bentley, O. G. and beattie, J. M.(eds.), 1958. Trace Elements, Academic Press, New York.

Langer, L. J. and Engel, L. L. 1958. In Boyer, P. D. et al.(eds.) The Enzymes, III. Academic Press, New York.

Lechartier, G. and Bellamy, F. 1877. In Lamb, C. A. et al.(eds.) 1958. Trace Elements. Academic Press, New York.

Leggett, C. F. and Boawn, I. C. 1963. Phosphate-induced zinc deficiency 14th Ann. Fert. Conf. of the Pacific North West 101-108.

Leyden, R. F. and Toth, S. J. 1960. Behavior of zinc sulfate as foliar application and as soil application in some New Jersey soils. Soil. Sci. 89:223-238

Linderstrom-lang, K. 1929, 1930. In Colowick, S. P. and Kaplan, N.O. Methods in Enxymology II, Academic Press, New York.

Lindskog, W. and Malmstrom, B. G. 1962. Metal binding and catalytic activity in bovine carbonic anhydrase. J. Biol. Chem. 237:1129

Lott, W. L. 1938. The relation of hydrogen ion concentration to the availability of zinc in soil. Soil Sci. Soc. Amer. Proc. 3:115-121

Mack, H. J., Fang, S. C. and Apple, S. B. 1964. Effect of soil temperature and phosphorus fertilization on snap beans and peas. Proc. Amer. Soc. Hort. Sci. 84:332-338

Mahler, H. R. Hubscher, G. and Baum, H. 1955. Studies on Uricase I. J. Biol. Chem. 216:625-641

Malvolta, E., Arzalia, J, and Hoag, H. 1956. Preliminary note on the absorption of radiozinc by young coffe plants.Phyton, 6:1-6

Martin, W. E., McLean, J. G. and Quick, J. 1965. Effect of temperature on the occurrence of phosphorus-induced zinc deficiency. Soil Sci. Soc. Amer. 29:411-413

Mathies, J. C. 1954. In Boyer, P. D. et al. (eds.) The Enzymes, III Academic Press, New York.

Maze', P. Influence of the different elements of the mineral solution on the development of maize. Ann. Inst. Pasteur, 28:21-68

McHargue, J. S. and Calfee, R. K. 1931. Effect of Mn, Cu and Zn on the growth and metabolism of Aspergillus flavus and Rhizopus nigricans. Bot. Gaz. 91:183-193

McHargue, L. S. and Calfee, R. K. 1931. Effect of Mn, Cu and Zn on the growth of yeast. Plant Phys. 6:559-566

Medina, A. and Nicholas, D. 1957. In Boyer, P. D. et al.(eds.) The Enzymes, III . Academic Press, New York.

Mendel, L. B, and Bradley, H. C. 1905. In. Underwood, E. J. Trace Elements Academic Press, New York.

Mirhij, J. I., 1968. Personal communication.

Mitchell, R. L. 1954. Trace elements and liming. Scot. Agr. 34:139-143

Nason, A., Kaplan, N.O. and Colowick, S. P. 1951. Changes in enzymatic constitution in zinc-deficient Neurospora. J. Bio. Chem. 188:397-406

Negelein, E. and Wulff, H. 1937. In Boyer, P. D. et al.(eds.) The Enzymes, III Academic Press, New York.

Neilands, J. B. 1957. Some aspects of microbial iron metabolism. Bacterial Revs. 21:101-111

O'Dell, B. L. 1932. In Underwood, E. J. Trace Elements, Academic Press, New York.

Ormrod, N. 1943. Zinc poisoning in rabbits. Vet. Rec. 55:366.

Patek, A. J. and Haig, C. 1939. In Lamb, C. A. et al.(eds.) 1958. Trace Elements. Academic Press, New York.

Piper, C. S. 1941., 1942. In Hewitt, E. J., 1966. Sand and Water Culture Methods Used in the Study of Plant nutrition., Commonwealth Agricultural Bureaux, Farnham, Bucks, England.

Plocke, D. J., Levinthal, C. and Vallee, B. L. 1962. Alkaline phosphatase of Escherichia coli, a zinc metalloenzyme. Biochemistry (Wash.) 1:373

Porges, N. 1932., Chemical composition of Aspergillus niger as modified by zinc sulfate. Bot. Gaz. 94:197-205

Possingham, T. V. 1956. In Lamb, C. A. et al.(eds.) 1958. Trace Elements. Academic Press, New York.

Prasad, A. S. 1966. Zinc Metabolism, Charles C. Thomas Publisher, Illinois, U.S.A.

Price, C. A. 1961. A zinc dependent lactate dehydrogenase in Euglena gracilis. Biochem. J. 82:61-66

Price, C. A. and Quigley, J. W. 1966. A method for determining quantitative zinc requirements for growth. Soil Sci. 101:11-16

Quinan-Watson, T. A. 1951. In Dixon, M. and Webb, E. 1958. Enzymes, Longmans, Green and Co., London.

Raulin, J. 1869.Etudes chimiques sur la vegetation. Ann. Sci. Nat. Bot. ser. 5, 11:93-299

Raulot, F. and Breton, H. 1877. In Lamb, C. A. et al.(eds.) 1958. Trace Elements. Academic Press, New York.

Reed, H. S. 1944. The growth of ovules of Pisum in relation to zinc. Am. J. Botany, 31:193-199

Riceman, D. S. 1948. In Hallsworth, E. G. Nutrition of the legumes. Butterworths Scientific Publications, London.

Riceman, D. S. and Jones, G. 1960. Distribution of recently absorbed zinc in subterranean clover (Trifolium subterraneum) determined by adding radioactive zinc to the culture solution. Aust. J. Agric. Res. 11:887-893

Rogers, S. H., Gall, O. E. and Barnett, R. M. 1939. The zinc content of weeds and volunteer grasses and planted covers. Soil Sci. 47:237-243

Rutter, W. J. and Ling, K. H. 1958. The mechanism of action of fructose diphosphate aldolase. Biochim. Biophys. Acta, 30:71

Sadasivan, V. 1950. Biochemical studies on Penicillium Chrysogenum Q.176 I phosphatase activity and the role of zinc in the production of penicillin. Arch. Biochem. 28:100-110

Sayre, J. D. 1952. Accumulation of radioisotopes in corn leaves. Ohio Agr. Exp. Sta. Research Bull. 723:1-23

Scharrer, K. and Jung, J. 1957. In Hewitt, E. J., 1966. Sand and Water Culture Methods Used in the Study of Plant nutrition., Commonwealth Agricultural Bureaux, Farnham, Bucks, England.

Scheltinga, H. 1954. Zinc in soil, plant and animal. Soils Sand Fert. 18:823

Schneider, K. H. and Siegel, O. 1958. Investigations by means of radioactive zinc on the zinc uptake by Aspergillus niger in calcareous soils. Soils and Fert. 22:1056, (1959)

Scott, D. A. and Mendive, J. R. 1941. Chemical observation on carbonic anhydrase. J. Biol. Chem. 140:445-450

Shkol'nik, M. and Abdurashitov, S. 1961. Effect of Trace Elements on the development of corn and on oxidation-reduction Processes during its ontogeny & Soviet Plant Phys. 8:No. 4:337-343

Skoog, F. 1940. Relationships between zinc and auxin in the growth of higher plants. Am. J. Botany 27:939-951

Smith, M. E. and Bayliss, N. S., 1942. The necessity of zinc for Pinus radiata. Plant Phys. 17:303-310

Smith, P. F. and Specht, A. W. 1952. Heavy metal nutrition an iron chlorosis of citrus seedlings. Plant Phys. 28:371-382

Sommer, A. L. 1927. The search for elements essential in only small amounts for plant growth. Science 66:487-484

Sommer, A. L. 1928. Further evidence of the essential nature of zinc for the growth of higher green plants. Plant Phys. 3:217-221

Sommer, A. L. and Lipmann, C. B. 1926. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Phys. 1:231-249

Sprague, H. B. (ed.), 1946. Hunger Signs in crops, 3rd ed. David McKay Co., New York.

Starkey, R. L. 1955. Relations of micronutrients to development of microorganisms. Soil Sci. 79:1-14

Steinberg, R. A. 1919. A study of some factors in the chenical stimulation of the growth of Aspergillus niger. Amer. Jour. Bot. 6:330-372

Steinberg, R. A. and Jeffrey, R. N. 1956. Effect of micronutrient deficiencies on nicotine formation by Tobacco in water culture. Plant Phys. 31:377-382

Stevenson, J. W. and Earle, I. P. 1956. In Lamb, C. A. et al.(eds.) 1958. Trace Elements. Academic Press, New York.

Steward, F. C. (ed.) 1963. Plant Physiology III, Academic Press, New York.

Stiles, W. 1961. Trace Elements in Plants. Cambridge University Press.

Stirn, F. E. Elvehjem, C. A. and Hart, F. B. 1935. The indispensability of zinc in the nutrition of the rat. Jour. Bio, Chem. 109:347-359

Stumpf, P. K. 1945. In Truog, E. (ed.) Mineral Nutrition of Plants. The University of Wisconsin Press. U. S. A.

Sudia, T. W. and Linck, A. J. The absorption and translocation of Zn-65 in Pisum sativum in relation to fruit development. Plant and soil 19:249-254

Sumner, J. and Somers, G. 1943. Chemistry and Methods of Enzymes. Academic Pres, New York.

Swaine, D. J. 1955. The Trace Element Content of Soils, Commonwealth Agricultural Bureaux, England.

Theorell, H. and Bonnichsen, R. 1951. In Boyer, P. D. et al. (eds.) The Enzymes, III. Academic Press, New York.

Thomas, T. 1965. The effect of a variation of zinc and protein in the diet on hair formation, zinc content of the hair and body growth of the albino rat. Unpublished.

Todd, W. R., Elvehjem, C. A. and Hart, E. B. 1934. Zinc in the nutrition of the rat. Amer. Jour. Physio. 107:146-156

Truog, E. 1948. Lime in relation to availability of plant nutrients. Soil Sci. 65:1-7

Truog, E. 1951. Mineral nutrition of Plants., The University of Wisconsin Press, U. S. A.

Tsui, C. 1948. The role of zinc in auxin synthesis in the tomato. Amer. Jour. Bot. 35:172-179

Tsuru, E., McConn, J. D. and Yasunobu, K. T. 1964. Bacillus subtilis neutral proteinaseI, a zinc enzyme of high specific activity Biochem. Biophys. Rec, Commun. 15:367

Trubowitz, S. Feldman, D, Morgenstern, S. W. and Hunt, V. M. 1961. The isolation, purification and some properties of the alkaline phosphatase of human leukocytes. Biochem. J. 80:369

Underwood, E. J. 1962. Trace Elements, Academic Press Inc., New York

Vallee, B. L. 1955. In Boyer, P. D. et al.(eds.) The Enzymes, III Academic Press, New York.

Vallee, B. L., Coombs, T. L. and Williams, R. J. 1957. In Lamb, C. A. (eds.) 1958. Trace Elements. Academic Press, New York.

Vallee, B. L. and Gilson, J. B. 1948. In Lamb, C. A., Bentley, O. G. and Beattie, J. M.(eds.) 1958. Trace Elements. Academic Press, New York.

Vallee, B. L. and Hoch, F. L. 1955. In Boyer, P. D. et al.(eds.) The Enzymes, III . Academic Press, New York.

Vallee, B. L., Hoch, F. L. and Adelstein, S. L. 1956, In Lamb, C. A. Bentley, O. G. and Beattie, J. M.(eds.) 1958. Trace Elements. Academic Press, New York.

Vallee, B. L. and Neurath, H. 1955. In Boyer, P. D. et al.(eds.) The Enzymes, III. Academic Press, New York.

Vallee, B. L. and Neurath, H. 1964. Carboxypeptidase, a zinc metalloprotein. J. Amer. Chem. Soc. 76:5006

Vallee, B. L. and Wacker, W., 1958. Zinc, a component of rabbit muscle dehydrogenase. J. Amer. Chem. Soc. 78:1771

Viets, F. G. 1951. Zinc deficiency of corn and beans on newly irrigated soils in Central Washington. Agron. J. 43:150-151

Viets, F. G., Boawn, L. C. and Crawford, C. L. 1954. Zinc content of bean plants in relation to deficiency symtoms and yield. Plant Phys. 29:76-79

Voelcker, J. A. 1913. Pot culture experiments. Jour. Roy. Agr. Soc. England, 74:411-422

Von Wartburg, J. P., Bethune, J. L. and Vallee, B. L. 1964. Human liver alcohol dehydrogenase. Kinetic and physicochemical properties. Biochemistry (Wash.) 3:1775

Wallace, A. 1963. Solute uptake by intact plants. Edwards Brother, Inc., Ann. Arbor. Michigan.

Wallihan. E. F. and Heymann-Herschberg, L. 1956. Some factors affecting absorption and translocation of zinc in citrus plants. Plant Phys. 31:294-299

Wang, C. H. and Willis, D. L. 1965. Radiotracer Methodology in Biological Science. Princeton Hall Inc., Englewood Cliffs, New Jersey.

Wanner, H. 1948. In Truog . Lime in relation to availability of plant nutrients. Soil Sci. 65:1-7

Warburg, O. and Christian, W. 1941. In Boyer, P. D. et al.(eds.) The Enzymes, III. Academic Press, New York.

Warburg, O. and Christian, W. 1943, In Steward, F. C.(ed.) Plant Physiology, III Academic Press, New York.

Watson, D. G, Davies, J. and Hanson, W. C. 1961. Zinc-65 in marine organisms along the Oregon and Washington coasts. Science 133:1826-1828.

Wear, J. I. 1956. Effect of soil pH and calcium on uptake of zinc by plants. Soil Sci. 81:311-315

White, A., Handler, P, Smith, E. and Stetten, D. 1959. Principles of Biochemistry. 2nd ed. McGraw-Hill Book Co. Inc., New York.

William, C. H. and Moore, C. W. 1952. The effect of stage of growth on the Cu,,Zn, Mn and Mo contents of Algerian Oats grown on 13 soils. Aust. J. Agr. Res. 3:343-361

Winder, F. and O'hara, C. 1961. Levels of some metabolites in normal, in iron-deficient, and in zinc-deficient Mycobacterium smegmatis. Biochem. J. 80:20p.

Winder, F. and O'hara, C. 1962. Effect of -P and of -Zn on the composition of Mycobacterium smegmatis. Biochem.J.82:98-108

Winder, F. and O'hara, C. 1966. Levels of iron and zinc in Mycobacterium smegmatis grown under conditions of trace metal limitation Biochem. J. 100:38p.

Wintersberger, E., Cox, D. and Neurath, H. 1962. Bovine pancreatic procarboxypeptidase B, I, Isolation, properties and activation. II-Mechanism of activation. Biochemistry (Wash.) 1:1069 & 1078

Wood, J. G. and Sibley, P. M. 1950. The distribution of zinc in oat plants. Aust. J. Sci. Res. 3:14-29

Wybenga, J. M. 1957, Sodium, A contribution to the knowledge of the importance of sodium for plant life. Kantoordruk De Goede-Wageningen.

Yudkin, W. H. and Fruton J. S. 1947. The activation of dehydropeptidase by zinc. Jour. Biol. Chem. 170:421-422

Plate 1.

Map of Lebanon showing the four areas from which soil samples were collected.

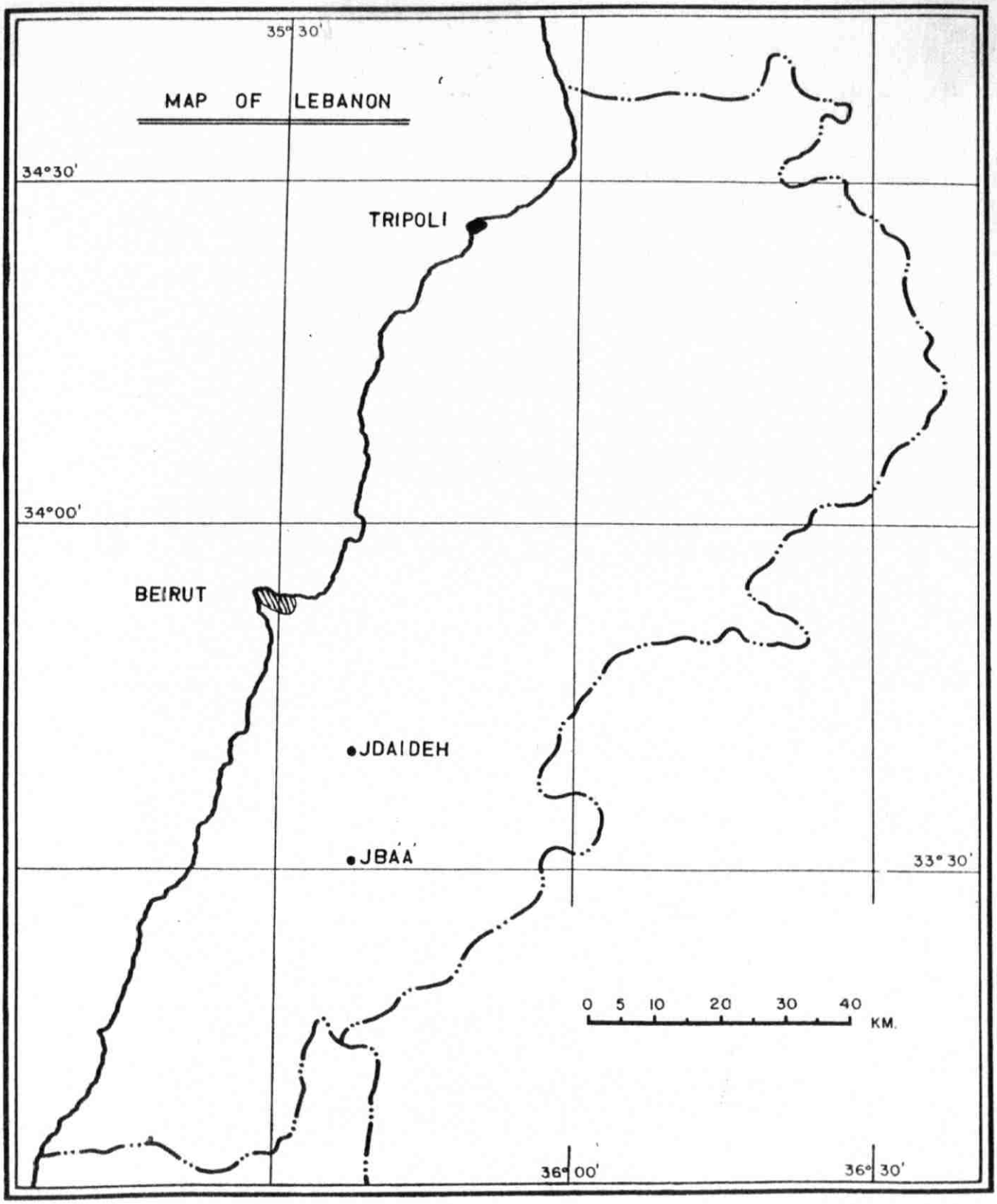

Plate 1. Sources Of Soil Samples

Plate 2.

Autoradiogram of a Pinto bean seedling grown in nutrient solution containing 14 ucu of Zn^{65}/liter for one week. Duration of exposure: 15 days.

Plate 3.

Autoradiogram of a Pinto bean seedling injected with 0.7 ucu of Zn^{65} in the petiole of one primary leaf. The plant was kept in the nutrient solution for 24 hours. Duration of exposure: 15 days.

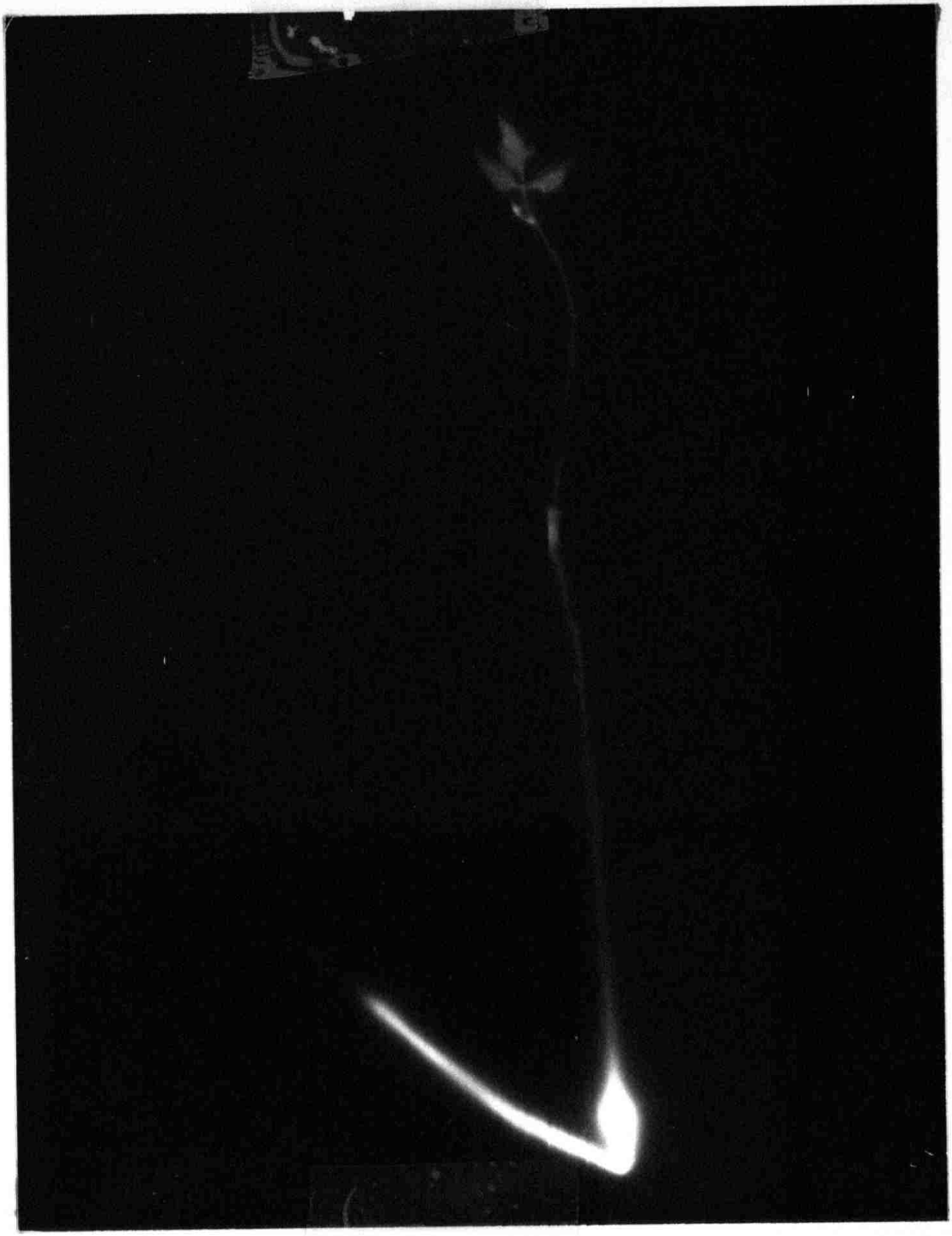

Plate 4.

Autoradiogram of a Pinto bean seedling injected with 0.7 ucu of Zn^{65} into the stem between the scars of the two cotyledons. The plant was kept in the nutrient solution for 24 hours. Duration of exposure: 15 days.

Plate 5.

Autoradiogram of a Pinto beans seedling injected with 0.7 ucu of Zn^{65} into the base of the stem. The plant was kept in the nutrient solution for 24 hours. Duration of exposure: 15 days.

APPENDIX A

Physical and chemical properties of zinc.

Periodic classification	Transition elements, zinc subgroup.
Atomic number	30
Atomic weight	65.37
Color	Bluish-white
Electron distribution	2,8,18,2
Melting point	419.5°C.
Boiling point	907°C.
Density (at 20°C.)	7.14 gm/cc.
Ionization potential	9.39 EV
Oxidation potential (to Zn^{++})	+0.76 volt

Radioactive zinc-65

Half-life	250 days	Prasad, 1966
	245 days	Francis et al. 1959
	244 days	Wang & Willis 1965
Mode of decay		
B^+(positron)	0.32 Mev	Prasad, 1966 Comar, 1955
	0.325 (2%) (1.7%)	Francis et al. 1959 Wang & Willis, 1965
γ (gamma ray)	1.44 Mev	Prasad, 1966
	1.11	Francis et al. 1959
	1.14	Wang & Willis, 1965
EC (Electron conversion)		
	0.009 (98%) (98.3%)	Francis et al. 1959 Wang & Willis 1965
Final product	Cu^{65}	Wang & Willis 1965

APPENDIX B

Binomial nomenclature of organisms referred to in the text.

Aspergillus flavus Link,

Aspergillus niger van Tieghem,

Baillus cereus Frankland and Frankland,

Coffea arabica L.,

Escherichia coli Castellàni and Chalmers,

Euglena gracilis Klebs,

Fagopyrum esculentum Moench,

Helianthus annuus L.,

Hordeum vulgare L.,

Lycopersicon esculentum Mill.,

Mycobacterium smegmatis Alvares and Tavel,

Neurospora crassa Shear and Dodge,

Penicillium chrysogenum Thom.,

Phaseolus vulgaris L.,

Pinus radiata Don.,

Pisum sativum L.,

Proteus vulgaris Hauser,

Rhizopus nigricans Ehrenberg,

Syotypus

Trifolium subterranium L.,

Ustilago sphaerogena Burrill,

Vicia faba L.,

Zea mays L.,