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ABSTRACT

Wheat (Triticum aestivum L.) grains, soybean (Glycine max (L.) Merr.) and tomato (Lycopersicon esculentum Mill.) seeds were germinated in Hoagland's nutrient solution #2 containing uranium concentrations ranging from 0.42 to 84.80 $\mu\text{gU/mL}$. Germination was unaffected but the subsequent growth was depressed in all the species. The total (protein) nitrogen content was unchanged in 6 or 7 d old seedlings, while it was decreased in the 10 d old seedlings. Tomato proved to be more sensitive to uranium toxicity than soybean or wheat.

Linear uptake of uranium was observed in the seedlings following germination, and uranium was preferentially accumulated in the shoot-root axes. Biosorption isotherms used to evaluate the biosorptive uptake capacity of the seedlings indicated that biosorption may be preceded by physico-chemical adsorption processes. At a solution pH 5.0 and 6.0 accumulation of uranium was maximized, while additional Ca^{2+} decreased uranium accumulation.

Uranium concentrations $\geq 42.40 \mu\text{gU/mL}$ decreased the influx and increased the efflux of K^+ in all the three species as determined by measuring K^+ in the germinating solution. In wheat roots, the accumulation of K^+ was decreased by 45% following exposure to uranium concentrations $\geq 42.40 \mu\text{gU/mL}$ determined by tissue analysis. This was paralleled by a significantly greater accumulation of 477% and 563% Na^+ over the control sets following exposure to 42.40 and 84.80 $\mu\text{gU/mL}$ respectively. The ratio of $\text{K}^+ : \text{Na}^+$ in the control set was 3.5:1. By contrast, in the uranium treated sets a reversed ratio of 1:2.4 and

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1:3.0 were observed respectively. At the same time, the accumulation of Mg^{2+} was decreased by 35 to 45% following exposure to 42.40 and 84.80 $\mu gU/mL$. The total ATP content of wheat roots was depressed by 40 to 75% by uranium treatments $\geq 4.24 \mu gU/mL$.

Scanning electron micrographs of wheat roots exposed to 84.80 $\mu gU/mL$ revealed a reduction in the length of the root hairs and lateral roots while there was no evidence of uranium deposition on the external root surface. Transmission electron micrographs showed relatively electron dense cell walls, plasmalemma dilations and invaginations leading to pinocytotic vesicles derived from the plasmalemma.

The relevance of this phytotoxic study to the field conditions is discussed.

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RÉSUMÉ

Des grains de blé (Triticum aestivum L.), des grains de soya (Glycine max (L.) Merr.) et de tomates (Lycopersicon esculentum Mill.) furent germés dans une solution nutritive (Hoagland's #2) contenant des concentrations d'uranium s'étendant de 0.42 à 84.80 µgU/mL. La germination ne fut pas affectée, mais la croissance subséquente fut inhibée chez toutes les espèces. Le contenu total d'azote ne changea pas chez les plantules âgées de 6 ou 7 jours, cependant il diminua chez les plantules âgées de 10 jours. Les tomates furent plus sensibles à la toxicité de l'uranium que le soya ou le blé.

Après la germination, une incorporation linéaire d'uranium fut observée chez les plantules et l'uranium fut préférentiellement accumulé dans l'axe pousse-racine. Des isothermes de biosorption, utilisés pour évaluer la capacité d'incorporation biosorptive, ont indiqué que la biosorption pouvait se faire par des processus d'absorption physico-chimiques. Aux pH de 5.0 et 6.0, l'accumulation d'uranium était maximisée, cependant l'addition de Ca^{2+} diminua l'accumulation d'uranium.

L'évaluation du K^+ dans la solution de germination indiqua que des concentrations d'uranium ≥ 42.40 µgU/mL ont diminué l'arrivée et augmenté le débit de K^+ chez les trois espèces. L'analyse des tissus détermina que l'accumulation de K^+ a diminué de 45% dans les racines de blé, après exposition à des concentrations d'uranium ≥ 42.40 µgU/mL. En même temps, une accumulation très significative de 477% et 563% de Na^+ par rapport aux plants témoins fut observée, à la suite de l'exposition à 42.40 et 84.80 µgU/mL respectivement. Le rapport $\text{K}^+:\text{Na}^+$ chez les

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témoins, était 3.5:1. Au contraire, chez les plantules exposées à l'uranium, des rapports inverses de 1:2.4 et 1:3.0 furent observées respectivement. En même temps, l'accumulation de Mg^{2+} diminuait de 35 à 45% après l'exposition des plants à 42.40 et 84.80 $\mu gU/ml$. Le contenu total d'ATP, dans les racines de blé, diminua de 40 à 75% à la suite de traitements à l'uranium $\approx 4.24 \mu gU/ml$.

Des microphotographies de racines de blé exposées à 84.80 $\mu gU/ml$ et obtenues à l'aide du microscope électronique à balayage ont mis en évidence une réduction de la longueur des poils absorbants et des racines latérales. Des microphotographies électroniques à transmission ont montré une paroi cellulaire très dense électroniquement, des dilations du plasmalemme et des invaginations qui deviendront des vésicules pinocytiques.

L'importance de cette étude phytotoxique par rapport aux conditions qui existent sur le terrain est discutée.

ABBREVIATIONS

ATP	-	adenosine triphosphate
ATPase	-	adenosine triphosphatase
Ci	-	curie
cv	-	cultivar
fCi	-	femtocurie (10^{-15})
$\mu\text{gU/mL}$	-	microgram per millilitre
pCi	-	picocurie (10^{-12})
R	-	rontgen
U	-	Uranium

1. INTRODUCTION

1.1. Radionuclide Contamination

Attention has recently been focused on the potential toxicological effects of radionuclides on plant growth. The contamination of the environment by radionuclides has been associated with nuclear electric power generation and the testing of nuclear weapons (Perkins and Thomas, 1980). In fact, a greater hazard may be due to the widespread use of phosphate rock and slag (containing radionuclides of the uranium-thorium decay series) which are widely used in phosphate fertilizers or in road building (Guimond, 1978; Melville et al., 1981). These radionuclides may be directly accumulated and recycled in the exposed biota or leached into the ground waters, and contaminate aquifers and aquatic environments distant from the site of application (Spalding and Sackett, 1972; Melville et al., 1981). Biologically, the most harmful and longest lived radionuclides are those belonging to the actinide series. The ecotoxicology of this group of chemicals has received little attention. The contamination may result in the accumulation of these elements in crop plants either by means of external deposition onto the plant leaf or from root uptake and transport to the edible parts. The root uptake is considered to be the major source of accumulation (Nishita et al., 1961; Romney et al., 1963; Dahlman et al., 1976; Romney and Wallace, 1977).

Most of the published biological studies on actinides have concentrated on plutonium, due to its presence in the environment from nuclear weapon testing (Francis, 1973; Price, 1973; Dahlman et al., 1976; Nishita et al., 1981). Other radionuclides such as uranium, thorium, americium, curium and neptunium have been studied mainly with reference to their comparative bioaccumulation potential under field conditions (Schreckhise and Cline, 1980; Garten, 1980; Garten et al., 1981; Schulz et al., 1981; Nishita et al., 1981; Romney et al., 1981). The studies on the comparative bioaccumulation of these radionuclides indicated that uranium, americium and curium accumulate in plants and animals more easily than plutonium (Bondietti et al., 1979; Garten, 1980; Garten et al., 1981).

1.2. Uranium Contamination

With the increased emphasis on nuclear energy, the environmental concentration of the radionuclide uranium has risen due to the mining and processing of uranium ores and runoff from tailings. Co-incidentally there has also been an increase in the use of phosphate fertilizers containing radionuclides belonging to the uranium-thorium decay series.

1.2.1. Uranium Contamination From Nuclear Energy

Much of the natural environmental radiation to which terrestrial organisms are exposed, is due to the radionuclides of uranium-thorium decay series (Garner, 1972). Hoffman and Kaye (1976) have pointed out that the release of uranium to the terrestrial environment is associated

with the operation of facilities used to prepare and reprocess radioactive fuel in U/Pu and Th/U nuclear fuel cycles. The environmental impact of operating nuclear power plants includes uranium mining, milling, refining, fuel fabrication, and fuel reprocessing, as well as waste storage (Schultz and Whicker, 1980). When the uranium is removed from the ore, radioactive daughters are left in the liquid and solid waste, (Schultz and Whicker, 1980). The processing and fuel fabrication associated with the fuel cycle results in the release of uranium to the environment. For example, the total uranium isotope released from one of the liquid-metal fast breeder reactor fuel fabrication plants is estimated to be 1048 $\mu\text{Ci/yr}$ (U.S. Atomic Energy Commission, 1974). In addition, various other sources can contribute to the entry of uranium into the environment such as global fallout, fuel reprocessing or waste storage (Garten, 1978). Iyengar and Markose (1970) reported that the agricultural soils and the vegetables grown on the soil around an uranium mine accumulated large quantities of uranium. Concentrations of uranium ranging from 0.57 to 32.47 $\mu\text{gU/kg}$ and 1.55 to 55.50 $\mu\text{gU/kg}$ were reported from the soil and vegetables respectively. Even the water (contaminated by surface flow of effluents through tailings or operational drains) used for irrigating the agricultural land was shown to contain 1.33 to 5.02 $\mu\text{gU/l}$ (Iyengar and Markose, 1970). The uranium concentrations in the crop plants has been reported by Smith et al. (1981) based on their field study around an integrated nuclear complex comprising 5 nuclear reactors, 2 nuclear fuel chemical separation facilities, a fuel fabrication facility and a heavy water production unit. For example a winter wheat crop grown on the soil collected from

one of these separation facilities was shown to contain 5.45 ± 3.52 , 0.56 ± 0.13 and 6.33 ± 5.85 fCi/g dry wt U^{234} , U^{235} and U^{238} respectively.

1.2.2. Uranium Contamination From Phosphate Fertilizers

Many phosphate ores contain high concentrations of uranium and other radionuclides. All the phosphate fertilizers derived from these phosphate rocks have been shown to contain various concentrations of radionuclides (Guimond, 1978). Many phosphate rock deposits in Idaho have been reported to contain an average of $90 \mu\text{gU/g}$ with a maximum of $400 \mu\text{gU/g}$ (U.S. Geol. survey, 1978). The uranium concentrations in the phosphate rocks range from 3 to $400 \mu\text{gU/g}$ world wide (Mazor, 1963; Menzel, 1968). Even the soil around phosphate processing plants may be contaminated. One such contaminated site was shown to contain 6.9 to $41.0 \mu\text{gU/g}$ (Johnson et al. 1980). Melville et al. (1981) studying the radioactive contamination of soil and vegetation from some slag roadbeds have reported uranium concentrations ranging from 1.1 to $18.2 \mu\text{gU/g}$ in the soil, while the plants growing on this soil accumulated 4.2 to $13 \mu\text{gU/g}$.

During the processing of phosphate ores, the radioactivity may be concentrated in the slag by-product. Road ways constructed with the slag have been found to contain a high gamma radiation of $50 \mu\text{R/h}$, and a slag dump site up to $90 \mu\text{R/h}$ (U.S. Environ. Protection Agency, 1977).

The concentration of uranium in various rivers flowing into the Gulf of Mexico was examined by Spalding and Sackett (1972). Their studies showed an increase in the concentration of uranium over those

observed 20 years ago and this has been attributed to the runoff from nearby agricultural lands which had heavy applications of phosphate fertilizers. Guimond (1978) made a detailed study of the radioactivity of uranium present in the phosphate fertilizers produced in the United States in the year 1974. His studies showed that a radioactivity of 1050 Ci/yr from U^{238} was present in the phosphate fertilizers. The fertilizers applied to many crops such as potatoes, sugarcane, tomato are 200 lb P_2O_5 /acre each year in the U.S. At an application rate of 250 lb P_2O_5 /acre, one application would result in the addition of 0.03 pCi U^{238} /g soil. The radioactivity of the agricultural soils in the U.S. typically ranges from 0.1 to 3 pCiU/g agricultural soil as a result of fertilizer application (Guimond, 1978). The rivers and lakes located near the agricultural lands probably receive the greatest amount of U^{238} from agricultural runoff (Guimond 1978). Hence, not only the crop plants grown in the agricultural land fertilized with phosphate fertilizers may be affected by uranium, but also the aquatic plants and animals exposed to uranium leached from watersheds distant from the site of agricultural application.

1.3. Uranium Chemistry:

Naturally occurring uranium (U_{nat}) is a mixture of three isotopes, U^{238} , U^{235} and U^{234} in a proportion of 99.28%, 0.71% and 0.006% respectively. The atomic weight of U_{nat} calculated by mass spectroscopy and nuclear data is 238.03. Chemical methods give the value of 238.07. In a new standard scale of atomic weights adopted in 1962 the atomic weight of U is 238.03. The isotope U^{238} is the longest lived

(4.50×10^9 years) and most abundant. U^{235} stands out among the natural isotopes because its nucleus is capable of nuclear fission. Eleven artificial isotopes of uranium have been obtained with mass numbers of 240, 239, 237, 236, 233, 232, 231, 230, 229, 228 and 227 (Palei, 1970).

The most important uranium bearing minerals are pitchblende (uraninite) with a composition close to U_3O_8 and carnotite which is a complex of uranyl potassium vanadate (Cotton and Wilkinson, 1980).

In both solid salts and solutions it occurs in 4 states of oxidation, $3+$, $4+$, $5+$ and $6+$. U^{3+} is very unstable in solution and is oxidized to U^{4+} . The U^{4+} state is fairly stable. In solution U^{5+} disproportionates with the formation of U^{4+} and U^{6+} . Hexavalent uranium is the most stable oxidation state (Bulman, 1980). The uranyl ion UO_2^{2+} is the basic form in which U^{6+} exists in solution. It possesses a linear configuration O-U-O (Cotton and Wilkinson, 1980). The ions UO_2^{2+} and U^{4+} are the most important species in the aqueous chemistry of uranium. The uranium oxides are UO_2 (brown black), U_3O_8 (greenish black) and UO_3 (orange yellow) (Cotton and Wilkinson, 1980).

Uranium is a fairly strong reducing agent and has a greater tendency to complex formation with most oxo-anions such as NO_3^- , SO_4^{2-} , CO_3^{2-} and PO_4^{3-} (Langmuir, 1978). Below pH 2.5 uranium (U^{6+}) exists in solution exclusively in the form of the uranyl ion UO_2^{2+} (Rothstein and Meier, 1951). Studies on the hydrolysis of uranium show that uranium forms mononuclear and polynuclear ions (Baes and Mesmer, 1976). The most probable mononuclear UO_2^{2+} hydrolysis species is $UO_2(OH)^+$ at $25^\circ C$ and it is known that nearly equal amounts of the unhydrolysed UO_2^{2+}

species and $(\text{UO}_2)_2(\text{OH})_2^{2+}$ occur in most acidic saturated solution.

At pH $\geq$ 5.0 the main uranium species is $(\text{UO}_2)_3(\text{OH})_5^+$ (Baes, and Mesmer, 1976; Bulman, 1980).

1.4. Terminology:

Uranium is often termed a heavy metal (Voegtlin and Hodge 1949/1953; Nechay et al., 1980; Horikoshi et al., 1981); Strandberg et al., 1981). The term 'heavy metals' is defined in the dictionaries of technical terms as those metals whose specific gravity is approximately 5 or higher (Lepedes, 1974), while Anon. (1964) includes metals with a specific gravity above 4. The main drawback of this classification is that it includes a heterogeneous array of elements with different chemical and biological properties. Since U^{238} which is a major component of natural uranium is an alpha emitter, the term "radionuclide" is used in the present thesis.

1.5. Uptake and Toxicity of Uranium:

1.5.1. Uptake of Uranium by Microorganisms:

The uptake of uranium and its effects on the metabolism of yeast cells have been extensively studied by Rothstein and co-workers (1948, 1948, 1951, 1954, 1956). Rothstein and Larrabee (1948) reported that the uptake of uranium in yeast cells proceeds in two phases, the first being a rapid phase followed by a slow phase and this uptake resulted in the inhibition of glucose metabolism. Rothstein et al., (1948) suggested

that the uranium formed dissociable complexes with certain active groups on the surface of the yeast cells, and later Rothstein and Meier (1951) concluded that the active complexing groups may include hydroxyl and carboxyl. They also pointed out that there are reactive groups on the yeast cell surface which are chemically similar to the high molecular weight polyphosphates and are responsible for complexing uranium. These complexes have been shown to be affected by pH, and are presumably involved in the transport of glucose into the cells (Rothstein and Meier, 1951). However, Barron et al., (1948) suggested that uranium complexes with proteins of the cell membrane thereby reducing the permeability for the transport of sugar. The studies of Rothstein and his group opened the way for the use of uranyl ions as potent inhibitors of carbohydrate utilization and the transport of amino acids in Baker's yeast (Kotyk et al., 1971).

Strandberg et al., (1981) reported that there is a surface associated accumulation of uranium by Saccharomyces cerevisiae. This was consistent with the view that uranium biosorption occurs by the formation of complexes with the active groups (eg. R-COO^- , PO_4^{2-}) on the cell surface. These findings clearly suggest that the membrane bound phosphate groups play a major role in uranium binding. Horikoshi et al. (1981) examined the uptake of uranium in several species of bacteria, yeast, fungi and the algae Chlorella. They reported that the biosorption isotherms followed the Freundlich equation suggesting that the uptake of uranium was mainly by physico-chemical adsorption. They also pointed out that the pH of the medium affected the uptake of uranium into these organisms.

1.5.2. Uptake of Uranium by Higher Plants:

The literature concerning the accumulation of uranium and its effects on the morphology of the plants comes mainly from the biogeochemical studies of Cannon (1952, 1957, 1971) who investigated the effects of ore deposits containing uranium and vanadium on the pine, rose and legume plants growing near the ore sites. She reported that large amounts of uranium accumulated in the rooting system and suggested that the metabolism of the plants may be affected by the unusual amounts of ore deposits in the roots. Subsequently Cannon (1971) showed that uranium is often associated with high concentrations of selenium, and selenium indicator plants may be used in prospecting uranium. Generally, large amounts of calcium, selenium and sulphur are absorbed along with uranium. Prister and Prister (1970) reported that greenhouse grown corn seedlings transferred to hydroponic solution containing 50 $\mu\text{gU/mL}$ died on the fourth day. Uranium had accumulated in the roots where most of the uranium was bound in a form incapable of exchange and only 10% was in the exchange absorbed state.

The natural concentration of uranium in various kinds of vegetables from three districts of Japan was shown to be in the range of 0.002 to 0.88 $\mu\text{gU/g}$ ash (Morishima et al., 1977). Sheppard et al., (1981) examined the concentration of uranium in 34 species of plants collected from three different areas on the Precambrian Shield (Bancroft and Bruin Lake, Edison Lake area of Ontario and Black Lake area of Manitoba). Several mines have operated in these areas and some are still used for uranium mining and milling. Pine, birch and spruce species were reported to contain 3 to 150 $\mu\text{gU/g}$ ash.

A limited number of isolated studies on the morphological effects of uranium on higher plants have been reported. However, other potentially toxic radionuclides (eg. thorium, arsenic) were also included in these studies. For example, the uptake and the effects of uranium, thorium and arsenic on Scot pine showed that large quantities of uranium were concentrated in the root system (root/soil concentration 346/26 $\mu\text{gU/g}$) resulting in decreased growth of the root system. This was related to the chemical toxicity of uranium to the root system leading to the reduction in water and mineral nutrient flow to the shoots (Sheppard and Thibault, 1980). Thibault and Sheppard (1980) reported that only 50% of the pine seedlings grown on Welcome Dump soil (containing 26 $\mu\text{gU/g}$, 5 $\mu\text{gTh/g}$ and 780 $\mu\text{gAs/g}$) survived after 120 days of exposure. The toxicity symptoms were shown to appear after 15 days of growth and were related to the chemical toxicity of uranium and arsenic (Thibault and Sheppard, 1980).

In addition, the accumulation of uranium in several lower and higher plant species has been observed (Dean, 1966; Edgington et al., 1970; Yamamoto and Masuda, 1974; Pribil and Marvan, 1976; Sakaguchi et al., 1978; Horikoshi et al., 1981).

Most of these previous studies have focused mainly on quantifying the concentration of uranium in soils and plant material. In addition, a few studies have reported the effects of other potentially toxic radionuclides (eg. thorium, arsenic) in combination with uranium on the growth of some plant species. No previous attempt has been made however, to evaluate the potential physiological effects

of uranium on crop plants likely to be exposed to the phosphate fertilizers of which 46 million tons are used annually in the U.S. and Canada (Stowasser, 1976).

1.6. Purpose of this Thesis:

Owing to its chemical toxicity and its radioactive nature, the increasing concentration of uranium recycled in the environment has raised increasing public concern. In view of the paucity of information available, it was deemed necessary to determine whether uranium at concentrations found in natural environments may be a perturbant factor during crop development. To this end, it was proposed to assess the physiological effects of uranium on the early stages of germination and growth of some crop species such as wheat, soybean and tomato which are a major source of food and are likely to be exposed to uranium contamination both from the nuclear industry and the phosphate fertilizers. Further, it was thought important to investigate the possible mode of uranium biosorption by these crop plants, and its mode of phytotoxicity.

1.7 Experimental Approach:

The initial hypothesis of the present work was that uranium is phytotoxic to the selected crop species.

To investigate this, the germination of the seeds and the morphology of the seedlings were studied under hydroponic conditions exposing the seeds and seedlings to a range of field relevant concentrations of uranium ranging from 0.42 to 84.80 $\mu\text{gU/mL}$.

Uranium accumulation and the influence of pH and the competing cation Ca^{2+} were studied. An attempt was made to determine the mode of biosorption of uranium by applying the Freundlich model equation of adsorption isotherms to the biosorption data. Further, such biochemical parameters as total plant protein and ATP content were determined under each level of treatment. The electrical conductivity of the rooting medium was monitored and specific K^{+} ion flux determined. These parameters were selected because they are indicative of seed and seedling vigor (Abdul-Baki, 1980). In addition, K^{+} , Na^{+} and Mg^{2+} accumulation in the seedling roots were analysed as a measure of membrane permeability.

Finally, scanning and transmission electron microscopic studies were made to assess the phytotoxicity of the highest uranium treatment at the cellular level.

2. MATERIALS AND METHODS

A. GENERAL METHODOLOGY

2.1 Chemicals:

Uranium oxide (natural uranium U_3O_8) was obtained from the Radiation Protection Bureau of National Health and Welfare, Ottawa, Canada. Atomic Absorption Standards (potassium, sodium and magnesium reference standard solutions $1000 \pm 1\% \mu\text{g/mL}$), toluidine blue, sodium hypochlorite, sodium hydroxide, silver nitrate, nitric acid, potassium sulphate, potassium persulfate, calcium chloride, sodium nitrate and mercuric oxide were purchased from Fisher Scientific Company, New Jersey, U.S.A. FF-ATP (adenosine triphosphate), FLE-50 buffered firefly lantern extract, and Trizma base were purchased from Sigma Chemical Company, Missouri, U.S.A. Glutaraldehyde, paraformaldehyde, osmium tetroxide, uranyl acetate, Spurr's low viscosity resin kit and copper grids were purchased from JB-EM Service Inc. Dorval, Quebec, Canada. Glass-distilled acetone was purchased from Caledon Laboratory Ltd., Georgetown, Ontario, Canada. All the chemicals used in the Hoagland's nutrient solution #2 were reagent grade and purchased either from Fisher Scientific Company, New Jersey, U.S.A., or Canlab Chemicals, Toronto, Canada.

2.2.1. Seed Material:

Soybean (Glycine max (L.) Merr. cv. Maplepresto), tomato (Lycopersicon esculentum Mill. cv. Ottawa 78) seeds and wheat (Triticum aestivum L. cv. Marquis Wheat) grains were donated by Agriculture Canada, Central Experimental Farm, Ottawa.

Before germination, the seeds and grains were surface sterilized in 2% sodium hypochlorite solution for 5 min and these were further rinsed with deionized distilled water for 10 min. The last water wash was tested with 1% silver nitrate solution to ensure that traces of hypochloride were no longer present.

2.2.2 Treatment of Seeds and Grains:

In parallel sets of experiments, 10 comparably sized soybean seeds, 10 wheat grains or 20 tomato seeds were separately placed in 9 cm pyrex petri-dishes, to each of which was added 20 mL Hoagland's nutrient solution #2 (Hoagland and Arnon, 1938) containing the uranium treatments of 0, 0.42, 0.84, 4.24, 8.48, 42.40 or 84.80 $\mu\text{gU/mL}$ corresponding to 0.5, 1.0, 5.0, 10.0, 50.0 or 100.0 $\mu\text{gU}_3\text{O}_8/\text{mL}$ (1 $\mu\text{gU}_3\text{O}_8/\text{mL}$ contains 0.848 $\mu\text{gU/mL}$).

All the control and treatment sets consisted of 10 petri-dishes representing a total sample size of 100 (soybean or wheat) or 200 (tomato) seeds and the experiments were repeated 3 to 5 times at different time intervals. The data were statistically analysed using one way analysis of variance (Tukey, 1949).

2.2.3. Germination:

The soybean and tomato seeds, and wheat grains were germinated in the dark under the following conditions: soybean; temperature $27 \pm 2^{\circ}\text{C}$, relative humidity 60% for 6 d, tomato; temperature $25 \pm 2^{\circ}\text{C}$, relative humidity 50% for 7 d, and wheat; temperature $30 \pm 2^{\circ}\text{C}$, relative humidity 40% for 6 d (the germination period for soybean and wheat was 6 d and 7 d for tomato).

The germinated seedlings were used to determine the percent germination and germination values, total seedling length, shoot and root length, percent protein nitrogen, percent mitotic division, and uranium uptake. In addition, the total ATP (adenosine triphosphate) content of the roots, and scanning and transmission electron microscopic studies were conducted with the roots of these seedlings. The germination solutions in the control and uranium treated sets were used to determine the spontaneous changes in pH, total electrolyte leakage, and specific ion leakage during the course of germination and early growth.

2.2.4. Statistical Analysis of Data:

Data collected from the experiments were analysed using an analysis of variance. Biomedical Computer program BMDP 2V developed by the Department of Biomathematics, U.C.L.A. and the Statistical Package for Social Sciences from SPSS Incorporated were used for analysis of variance. Significance was determined by Tukey's test (Tukey, 1949).

B. Uranium Phytotoxicity

2.3.1. Percent Germination and Germination Values:

Soybean and tomato seeds, and wheat grains were germinated as described in Section 2.2.3. During the course of 6 or 7 d germination, the seeds were examined daily to determine the onset of germination. Germination was assessed to be complete when the root pierced the seed coat (Mayer and Poljakoff-Mayber, 1963). The final germination was calculated as percent of the total number of seeds germinated per treatment set following the required 6 d incubation period for soybean or wheat, and 7 d for tomato. The germination values were computed from the mean daily germination and maximum daily germination or peak values (Czabator, 1962).

2.3.2. Seedling Length and Weight:

After 6 or 7 d germination, the lengths of the wheat seedlings were measured from the tip of the coleoptile to the tip of the root cap. The seedling lengths of soybean and/or tomato were measured from the shoot tip to the root cap. The measured seedlings were then weighed, dried in an oven at 100°C for 24 h, and weighed again.

2.3.3. Shoot and Root Length:

The shoot and root lengths of the soybean seedlings could not be separately measured as it was difficult to delineate the exact location of the transition between the base of the hypocotyl and the beginning of the root. The shoot length of wheat was measured from the tip of the coleoptile to the point of attachment to the endosperm, and of tomato, from the top of the shoot to the base of the hypocotyl. The root length of wheat was measured from the tip of the root to the point of attachment to the endosperm, and of tomato, from the tip of the root to the base of the hypocotyl.

2.4 Percent Protein Determination:

The total % nitrogen in the 6 or 7 d old seedlings and 10 d old seedlings of all the three crop species was determined by the microKjeldahl procedure.

The seedling samples were freeze-dried, weighed and transferred separately to 100 mL Kjeldahl digestion flasks. Potassium sulfate ($1.9 \pm 0.1\text{g}$), mercuric oxide ($40 \pm 10\text{ mg}$) and sulphuric acid ($5.0 \pm 0.1\text{ mL}$) were added to each of the digestion flasks. The flasks were then placed on a digestion rack and boiled for 45 min to 1 h until the initial dark color of the mixture disappeared and a clear solution was obtained. Following this, a minimum volume of deionized distilled water was added.

to dissolve any residual solid or precipitate. The digest was then transferred to the distillation flask. The digestion flask was rinsed and 15 to 20 mL NaOH/Na₂S₂O₃ solution added to the digested solution. An Erlenmeyer flask (25 mL) containing 5 mL saturated boric acid and 2-4 drops of indicator solution was placed under the condenser with the condenser tip below the surface of the receiving solution. The digested solution was distilled until about 15-20 mL of the distillate was collected. The Erlenmeyer flask was removed when the indicator changed color from purple to green. The volume of the distillate was made up to 50 mL with deionized distilled water and titrated against 0.02 N HCl to the grey end point. The nitrogen content of the seedling sample was calculated from the expression:

$$\%N = \frac{(\text{Sample titre} - \text{blank titre}) \times \text{Normality} \times 14.007 \times 100}{\text{Weight of the sample (mg)}}$$

The general protein factor 6.25 was used in the soybean and tomato sets to convert the weight of nitrogen in the sample to weight of protein, and the protein factor of 5.7 was used for wheat (Horwitz, 1980). These calculations will yield an overestimate of protein content since seedling tissues also contain non-protein nitrogen (free amino acids, amides, nitrate ions, nucleotides).

2.5. Percent Mitotic Division:

The roots of 6 d old wheat seedlings from control and 84.80 $\mu\text{gU/mL}$ uranium treated sets were harvested. Ten root tips were sectioned in each of the control and uranium treated sets. Two mm root tips were excised and prefixed in 2% formaldehyde - 2% glutaraldehyde in 0.05 M sodium phosphate buffer (pH 7.0) for 2 h. The root tip segments were washed with sodium phosphate buffer and post-fixed for 1 h in cold buffered aqueous 1% osmium tetroxide. The root tips were then dehydrated through an acetone series (50-100%) and infiltrated with Spurr's low viscosity resin mixture (Spurr, 1966). This was then polymerized at 65°C for 16 h. Semi-thin sections were cut with a glass knife on a Sorval Poster-Blum MT-2B ultramicrotome and then mounted on a glass microslide. The semi-thin sections were stained in 0.05% toluidine blue. The sections were observed under a light microscope to determine the percent mitotic division in the control and uranium treated sets. Five hundred cells (5 fields of 100 cells each) in each of the sections were counted to a total of 5000 cells.

2.6.1. Growth: Soybean:

In a parallel set of experiments, 4 d old soybean seedlings (germinated in deionized distilled water) were transferred to 250 mL Erlenmeyer flasks each containing 100 mL Hoagland's nutrient solution #2 (Hoagland and Arnon, 1938). Uranium oxide was added to give final concentrations of 0, 0.42, or 42.40 $\mu\text{gU/mL}$. The seedlings were then grown for a further 4 wk in an environmentally controlled growth chamber.

with a day:night temperature of 20:18°C, light:dark 12:12 h (17 Klx) and relative humidity 60%. The solutions were changed on days 6, 11, 15, 18, 22 and 26, and the pH of the media was maintained in the range of pH 5.0 to pH 5.6. Following this, the plants were harvested for chlorophyll and uranium analyses. The uranium content of the shoots and roots was separately analysed by laser fluorimetry (Measures and Lecompte, 1980).

2.6.2. Chlorophyll Analysis:

0.5 g of ontogenetically similar true leaves (L₁, L₂ and L₃) were homogenized in 8 mL of 98% acetone, filtered through Whatman #1 filter paper and diluted to 80% v/v acetone: deionized distilled water. Optical density readings were made at 663 and 645 nm using a Unicam SP 1800 Ultraviolet Spectrophotometer and total chlorophyll, chl_a and chl_b calculated (Arnon, 1949).

C. URANIUM UPTAKE

2.7.1. Uranium Analysis:

Six day old soybean or wheat seedlings were separated into shoot-root axes, and cotyledons of soybean or endosperm of wheat. Uranium was separately analysed in each of these organs. The tomato seedlings (7 d) were very fine and small, as a consequence the whole seedlings were analysed. The 4 wk old soybean seedlings were separated into shoots and roots.

All these samples (shoot-root axes and cotyledons or endosperm of 6 d old soybean or wheat seedlings, whole seedlings of 7 d old tomato, and shoot and roots of 4 wk old soybean plants) were dried at 100°C for 24 h. One gram dry wt. of each of the samples was dry ashed in a muffle furnace for 2 h at 650°C. The ashed residue was dissolved in 25 ml of concentrated nitric acid, 2 ml aliquot of this sample was diluted in 25 ml deionized distilled water and 2 g of potassium persulfate were added. The solution was then evaporated to dryness. The residue was dissolved in 40 ml deionized distilled water and the pH adjusted to pH 8.0 with 10 N sodium hydroxide and then neutralized with 10% nitric acid. The resultant solution was transferred to a 50 ml volumetric flask and diluted with deionized distilled water to 50 mL. The uranium content of the tissue in each set was determined by laser fluorimetry (Measures and Lecompte, 1980).

2.7.2. Mathematical Modelling:

The Freundlich model equation was applied to the uranium biosorption data of all the three species after a 6 or 7 d germination period to explain the physico-chemical adsorption of uranium (Freundlich 1926). Individual Freundlich equations were computed for each set of data.

2.7.3. Influence of Additional Calcium on Uranium Uptake:

In two parallel sets of experiments, 4 d old wheat seedlings were transferred to Hoagland's nutrient solution #2 containing 84.80 $\mu\text{gU/mL}$. An additional 100 $\mu\text{gCa}^{2+}/\text{mL}$ was added to one set. The seedlings of both sets were grown for a further 10 d under environmentally controlled conditions with day:night temperature 30:20°C, light:dark 16:8 h (17 Klx.) and 40% relative humidity. Ten days post-treatment, uranium accumulation into the 14 d old wheat seedlings of each set was analysed by delayed neutron activation analysis.

Briefly, the technique of neutron activation/delayed neutron counting involves irradiating an unknown sample for 60 s, transferring the sample to a counting facility and after 10 s delay period, counting the sample for 60 s. By comparing the delayed neutron count to that obtained from a standard or reference material, the uranium content of the unknown sample can be determined. As only U^{235} is fissioned by thermal neutrons, neutron activation/delayed neutron counting is specific for the determination of U^{235} or in fact uranium (assuming the normal isotopic abundance of 0.72% U^{235} in the natural uranium). The system developed and currently employed by Commercial Products of AECL has a sensitivity of 205 ± 4 counts per microgram of natural uranium with a detection limit of 0.1 μg uranium or 0.1 $\mu\text{g/g}$ for a 1 g (dry wt.) sample (Boulanger et al., 1975).

2.7.4. Influence of pH on Uranium Uptake:

In five further experimental sets, 4 d old wheat seedlings were exposed to 8.48 $\mu\text{gU/mL}$ and the media pH adjusted to pH 4.0, 5.0, 6.0, 7.0 or 8.0. The seedlings were grown for a further 10 d as described in Section 2.7.3. The solutions were changed on day 4 and 7. Ten days post-treatment, the total uranium content of the whole seedlings was analysed by delayed neutron activation (Boulanger et al., 1975).

2.8 Spontaneous Changes in pH of the Media:

Soybean and tomato seeds, and wheat grains were germinated as described in Section 2.2.3. The pH of the germinating media in which the seeds were germinated was determined on days 0 and 6 (soybean and wheat) or day 7 (tomato) using a pH meter (Orion Research Digital Ionalyser/501).

D. ION FLUX AND MEMBRANE PERMEABILITY

2.9 Solute Leakage:

The time course study of solute leakage from germinating seeds of all of the three species was determined by measuring the electrical conductivity of the germination media and by monitoring for specific ion leakage (K^+) during the course of germination (6 or 7 d), and subsequent growth (10 d) of the seedlings.

2.9.1. Electrical Conductivity Measurements:

The seeds of all three species were germinated as described previously (Section 2.2.3.). The electrical conductivity of the germination media was determined daily until germination was completed (6 or 7 d). In the sets containing wheat seedlings, the daily monitoring of electrical conductivity was extended for a further 4 d to a total of 10 d. The readings were taken with a CDM/2e conductivity meter with a dipcell type CDC 114.

2.9.2. Specific Ion Flux: Potassium:

The seeds of all the three species were germinated as described previously (Section 2.2.3.). The K^+ ion concentrations in the media were determined by Atomic Absorption Spectrophotometry. The time course of potassium ion flux was monitored daily in the germination solution of all the three crop species. A total of 9 replicates were used for each control and uranium treated sets. During the course of germination and early growth, 0.5 mL aliquots of the germinating media of each set were taken for the determination of K^+ . These aliquots were then made up to 25 mL with deionized distilled water. Each of these samples was then separately analysed for K^+ at a wavelength of 766 nm on a Varian AA-175 Atomic Absorption Spectrophotometer. Acetylene and air were used as fuel and oxidant in the flame. The concentrations of potassium in the media of all sets were quantified using a standard curve (Dean, 1960).

Monitoring of the K^+ content of the media was extended for a further 4 d in the sets containing wheat seedlings to follow the changes during early growth.

2.10. Total Root ATP Content:

The total ATP content of the roots of wheat seedlings exposed to a range of uranium concentrations (0, 4.24, 8.48, 42.40 or 84.80 $\mu\text{gU/mL}$) was determined following day 2, 4, 6, 8 and 10 d germination and early growth using the modified luciferin-luciferase method of Ching (1973). The wheat grains were germinated as described previously (Section 2.2.3.). Four replicates of 10 seedlings were harvested in each set at each experimental time period. The roots were excised from the seedlings and washed with deionized distilled water for 10 min, then immersed in 10 mL boiling Trizma buffer (pH 7.75) and ATP was extracted for 10 min. The extract was cooled in an ice bath. The extract, if not immediately used was kept frozen. Adenosine triphosphate (ATP) was assayed by mixing 0.5 mL of the extract with 1.5 mL of enzyme (firefly-lantern extract) for 60 s. The ATP content was determined as measurement of light emission during the oxidation of luciferin. The luminescence of the extract was determined using a Beckman L.S. 3133 P. liquid scintillation counter. A standard curve was obtained for ATP, and this was used to quantify the total ATP content in the samples (Patterson et al., 1970).

2.11. K⁺, Na⁺, and Mg²⁺ Accumulation:

In a parallel set of experiments, wheat grains were germinated in deionized distilled water for 6 d and transferred to Hoagland's nutrient solution #2 augmented with uranium oxide to give a final concentration of 0, 42.40 or 84.80 $\mu\text{gU/mL}$ in the media. The control and treatment sets consisted of 3 replicates of 10 seedlings and the

experiment was repeated at two different times. The concentration of sodium was adjusted to 6 mM using NaNO_3 . The seedlings were grown under environmentally controlled conditions as described (Section 2.7.3.). Ten days post-treatment, the wheat seedlings were harvested and the roots were excised and weighed (fresh wt.). These samples were dried to constant weight at 100°C for 24 h. The dried root material was dry ashed at 560°C for 48 h, digested in 0.5 mL concentrated nitric acid and then diluted to the required volume with deionized distilled water. The potassium and sodium concentrations in the samples were determined by flame photometry and the magnesium content by atomic absorption spectrophotometry. The readings were taken on a Jarrell Ash Atomic Absorption Spectrophotometer at a wavelength of 766 nm for the potassium determination. Sodium was assayed at 585 nm and magnesium at 285 nm. Hydrogen and air were used as fuel and oxidant in the flame photometry, and acetylene and air for atomic absorption spectrophotometry. Standard curves were obtained for K^+ , Na^+ and Mg^{2+} , and these were used to quantify the total ions in the samples (Dean, 1960).

2.12. Scanning Electron Microscopy:

SEM was performed to determine whether there was any evidence of uranium deposition on the root surface and the effects on the root length. The roots of 10 d old wheat seedlings from control and 84.80 $\mu\text{gU/mL}$ treated sets were fixed, dehydrated as described previously (Section 2.5.). Following dehydration in the acetone series (50-100%), the critical point drying was carried out under CO_2 in a Polaron

Critical Point Dryer. The specimens were mounted on stubs with silver paste and sputter coated with 60:40 gold:palladium mixture. The specimens were observed in a JEOL scanning electron microscope and photographed with a polaroid type-55 film.

2.13. Transmission Electron Microscopy:

The roots of 10 d old wheat seedlings were harvested, fixed, dehydrated and infiltrated as previously described (Section 2.5.). Thin sections were cut with a Dupont diamond knife on a Reichert Om U₂ ultramicrotome and then mounted on carbon coated copper grids. The sections were stained for 5 min with uranyl acetate (5% in 50% ethanol) and for 4 min in lead citrate (Reynolds, 1969).

Observations were made using a Philips 201 electron microscope and photographed on Kodak electron image film SO-163. This study was repeated following the method described above except that a glass knife was used for sectioning on a Sorval Porter Blum MT-2B ultramicrotome. The sections were mounted on acetone treated copper grids without support.

3. RESULTS

A. URANIUM PHYTOTOXICITY:

3.1.1. Percent Germination and Germination Values:

The total final percent germination and the germination values of wheat, soybean and tomato were not significantly affected by uranium concentrations within the range of 0.42 to 84.80 $\mu\text{gU/mL}$ media. (Appendices I, II and III).

3.1.2. Seedling Lengths:

The early growth of the seedlings, as opposed to germination, was significantly depressed in all the three species at concentrations 42.40 $\mu\text{gU/mL}$. Some species exhibited sensitivity at ≈ 4.24 $\mu\text{gU/mL}$. The order of sensitivity from least to most sensitive was wheat soybean tomato.

The seedling length of wheat was depressed by 12 and 22% by uranium concentrations 42.40 and 84.80 $\mu\text{gU/mL}$ respectively (Table 1).

Soybean seedlings were less toxitolerant (Table 2). At a 10 fold lower concentration of uranium (8.48 $\mu\text{gU/mL}$), a 22% decreased growth was observed. There was a 33% and 36% decrease in the length of soybean seedlings exposed to 42.40 and 84.80 $\mu\text{gU/mL}$ respectively.

Tomato seedlings were most sensitive to uranium treatments (Table 3). A depressed seedling growth of 24% was evident following 7 d exposure to 4.24 $\mu\text{gU/mL}$ concentration and a 25% reduction was obtained at 8.48 $\mu\text{gU/mL}$. The higher treatments (42.40 and 84.80 $\mu\text{gU/mL}$) evoked a higher toxicity response towards the growth of tomato seedlings evidenced by a significant 44% and 49% decrease in growth as compared to the control.

Fresh and dry weights (Appendices X, XI and XII) were not significantly different between the control and uranium treated seedlings in all the three species.

TABLE 1
Effect of uranium on seedling length (cm) of wheat (6 d)
after the completion of germination

Treatments $\mu\text{gU/mL}$	Group mean $\pm$ S.E.	% of control
Control	24.37 $\pm$ 1.37 a*	
0.42	25.73 $\pm$ 1.31 a	105
0.84	24.42 $\pm$ 1.34 a	100
4.24	24.53 $\pm$ 1.30 a	100
8.48	26.31 $\pm$ 1.34 a	107
42.40	21.55 $\pm$ 1.12 b	88
84.80	19.05 $\pm$ 1.06 b	78

* Means (n = 300) with standard error followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).

TABLE 2

Effect of uranium on seedling length (cm) of soybean (6 d)
after the completion of germination

Treatments μgU/mL	Group mean ± S.E.	% of control
Control	10.00 ± 0.46 a*	
0.42	9.30 ± 0.45 a	93
0.84	9.28 ± 0.46 a	91
4.24	9.15 ± 0.41 a	91
8.48	7.81 ± 0.40 b	78
42.40	6.75 ± 0.38 c	67
84.80	6.43 ± 0.31 c	64

* Means (n = 300) with standard error followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

TABLE 3

Effect of uranium on seedling length (cm) of tomato (7 d)
after the completion of germination

Treatments μgU/mL	Group mean ± S.E.	% of control
Control	9.72 ± 0.25 a*	
0.42	9.40 ± 0.26 a	97
0.84	9.22 ± 0.31 a	94
4.24	7.62 ± 0.24 b	76
8.48	7.43 ± 0.26 b	75
42.40	5.15 ± 0.21 c	56
84.80	4.99 ± 0.19 c	51

* Means (n = 300) with standard error followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

3.1.3. Shoot and Root Length:

The shoot length of wheat was unaffected by uranium treatments in the range of 0.42 to 84.80 $\mu\text{gU/mL}$ (Appendix IV). In the tomato seedlings, however, a 38 and 44% decrease in the shoot length was observed following exposure to 42.40 and 84.80 $\mu\text{gU/mL}$ respectively (Table 4).

The root length of wheat was reduced by 21% and 31% following 42.40 and 84.80 $\mu\text{gU/mL}$ treatment (Table 5).

Tomato was less toxitolerant to uranium exposure and a decrease in root length was noted at a low concentration of 4.24 $\mu\text{gU/mL}$ (Table 6). A 23% and 28% reduction in tomato root length was evidenced at 4.24 and 8.48 $\mu\text{gU/mL}$ respectively. Exposure to the higher concentrations of uranium, namely 42.40 and 84.80 $\mu\text{gU/mL}$ caused a highly significant 45% and 48% reduction in root growth as compared to the control tomato seedlings.

It is interesting to note that the seedling lengths of wheat and tomato were significantly reduced following exposure to 42.40 and 4.24 $\mu\text{gU/mL}$ respectively. However, in the case of wheat seedlings this could be attributed solely to the reduction in the root length, total length of the shoot (coleoptile) not being significantly affected (Appendix IV). In tomato, some shoot length reduction was noted, but only at concentrations 10-fold higher (42.40 and 84.80 $\mu\text{gU/mL}$) than those required to evoke the toxic response of the roots, namely 4.24 and 8.48 $\mu\text{gU/mL}$.

TABLE 4

Effect of uranium on shoot length (cm) of tomato (7 d)
after the completion of germination

Treatments $\mu\text{gU/mL}$	Group mean $\pm$ S.E.	% of control
Control	4.98 $\pm$ 0.17 a*	
0.42	5.95 $\pm$ 0.16 a	119
0.84	5.87 $\pm$ 0.26 a	117
4.24	4.78 $\pm$ 0.18 a	95
8.48	4.72 $\pm$ 0.18 a	94
42.40	3.12 $\pm$ 0.14 b	62
84.80	2.81 $\pm$ 0.12 b	56

* Means (n = 300) with standard error followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

TABLE 5

Effect of uranium on root length (cm) of wheat (6 d)
after the completion of germination

Treatments $\mu\text{gU/mL}$	Group mean $\pm$ S.E.	% of control
Control	15.73 $\pm$ 0.9 a*	
0.42	15.82 $\pm$ 0.8 a	100
0.84	15.74 $\pm$ 0.8 a	100
4.24	15.29 $\pm$ 0.8 a	97
8.48	15.22 $\pm$ 0.9 a	96
42.40	12.58 $\pm$ 0.7 b	79
84.80	10.91 $\pm$ 0.6 b	69

* Means (n = 300) with standard error followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).

TABLE 6
Effect of uranium on root length (cm) of tomato (7 d)
after the completion of germination

Treatments μgU/mL	Group mean ± S.E.	% of control
Control	3.84 ± 0.08 a*	
0.42	3.85 ± 0.08 a	100
0.84	3.66 ± 0.08 a	96
4.24	2.97 ± 0.09 b	77
8.48	2.75 ± 0.07 b	72
42.40	2.11 ± 0.06 c	55
84.80	2.00 ± 0.07 c	52

* Means (n = 300) with standard error followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

3.2. Percent (Protein) Nitrogen:

The total percent (protein) nitrogen of the 6 or 7 d old seedlings exposed to the range of uranium concentrations of 4.24 to 84.80 $\mu\text{gU/mL}$ was unaffected (Tables 7-9). However, during subsequent early growth, the protein nitrogen was depressed in all the three species. Again, the same specificity of uranium toxicity was noted. Tomato was the more sensitive and wheat the least sensitive.

The two lower concentrations of uranium namely, 4.24 and 8.48 $\mu\text{gU/mL}$ did not elicit any significant decrease in the (protein) nitrogen content of wheat seedlings. However, exposures to the two higher treatments (42.40 and 84.80 $\mu\text{gU/mL}$) caused a significant 29 and 31% decrease, representing 71 and 69% of the control (Table 7).

In soybean seedlings, the onset of uranium toxicity was observed at concentrations 8.48 $\mu\text{gU/mL}$. A significant 30 to 36% decrease in (protein) nitrogen was obtained following exposure to uranium concentrations between 8.48 to 84.80 $\mu\text{gU/mL}$ (Table 8).

TABLE 7

Effect of uranium on percent protein in 10 d old wheat seedlings

Treatments μgU/ml	% protein ± S.D.	% of control
Control	31 ± 1.5 a*	
4.24	28 ± 1.6 a	89 ± 5
8.48	27 ± 2.0 a	85 ± 6
42.40	22 ± 3.6 b	71 ± 9
84.80	22 ± 1.3 b	69 ± 4

* Means (n = 6), with standard deviation followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

TABLE 8

Effect of uranium on percent protein in 10 d old soybean seedlings

Treatments µgU/mL	% protein ± S.D.	% of control
Control	25 ± 2.4 a*	
4.24	20 ± 1.0 a	80 ± 5
8.48	17 ± 0.4 b	70 ± 2
42.40	17 ± 0.5 b	67 ± 2
84.80	16 ± 0.4 b	64 ± 2

* Means (n = 6) with standard deviation followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

In tomato seedlings, (Table 9) toxicity of uranium was evident following exposure to the relatively low concentration of 4.24 $\mu\text{gU/mL}$. Treatments of 4.24 and 8.48 $\mu\text{gU/mL}$ evoked similarly significant moderate effects, whereas 42.40 and 84.80 $\mu\text{gU/mL}$ elicited significantly severe reduction in total protein. A significant 9 to 10% decrease in (protein) nitrogen was observed in the seedlings exposed to 4.24 and 8.48 $\mu\text{gU/mL}$ and a 17 to 20% decrease in the seedlings exposed to 42.40 and 84.80 $\mu\text{gU/mL}$.

TABLE 9

Effect of uranium on percent protein in 10 d old tomato seedlings

Treatments μgU/mL	% protein ± S.D.	% of control
Control	28 ± 0.7 a*	
4.24	25 ± 0.4 ab	91 ± 1.5
8.48	25 ± 0.6 ab	90 ± 2.0
42.40	23 ± 1.4 b	83 ± 4.5
84.80	22 ± 1.4 b	80 ± 4.7

* Means (n = 6) with standard deviation followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

3.3. Percent Mitotic Division:

Six days post treatment, the total percent mitotic division of wheat root tip meristem cells was significantly decreased by 28% following exposure to 84.80 $\mu\text{gU/mL}$ (Table 10). However, no other changes were observed in the nucleus.

3.4.1. Growth of 4 wk old Soybean Plants:

The toxic symptoms induced by uranium in 4 wk old soybean plants included a general reduction in the root growth, chlorosis of leaves, a wide-spread necrosis and early leaf abscission at a concentration of 42.40 $\mu\text{gU/mL}$ treatment (Figure 1). Visual observations indicated that the toxic symptoms appeared only after 3 wk growth.

3.4.2. Chlorophyll Content:

The effects of uranium (0.42 and 42.40 $\mu\text{gU/mL}$) on the total chlorophyll, Chl_a and Chl_b expressed as percentage of control are shown in Table 11.

In plants exposed to 0.42 $\mu\text{gU/mL}$, the total chlorophyll, Chl_a and Chl_b were not significantly different from the control sets in any of the leaves. However, a 30 to 40% reduction in total chlorophyll, chl_a and chl_b was observed in leaves 2 and 3 exposed to 42.40 $\mu\text{gU/mL}$.

TABLE 10

Effect of uranium (84.80 μ gU/mL) on the % mitotic division in the wheat root tip meristem 6 d post treatment

Treatments μ gU/mL	% mitotic division Mean $\pm$ S.D.	% of control
Control	10.5 $\pm$ 2.6 a*	
84.80	7.6 $\pm$ 1.1 b	72 $\pm$ 10

* Means (n = 50) with standard deviation followed by the same letter is not significantly different by Student t-test at $P < 0.05$ level.

FIGURE 1

Effects of uranium on four week old soybean plants. Right to Left:
Control, treated with 0.42, and 42.40 ugU/mL.

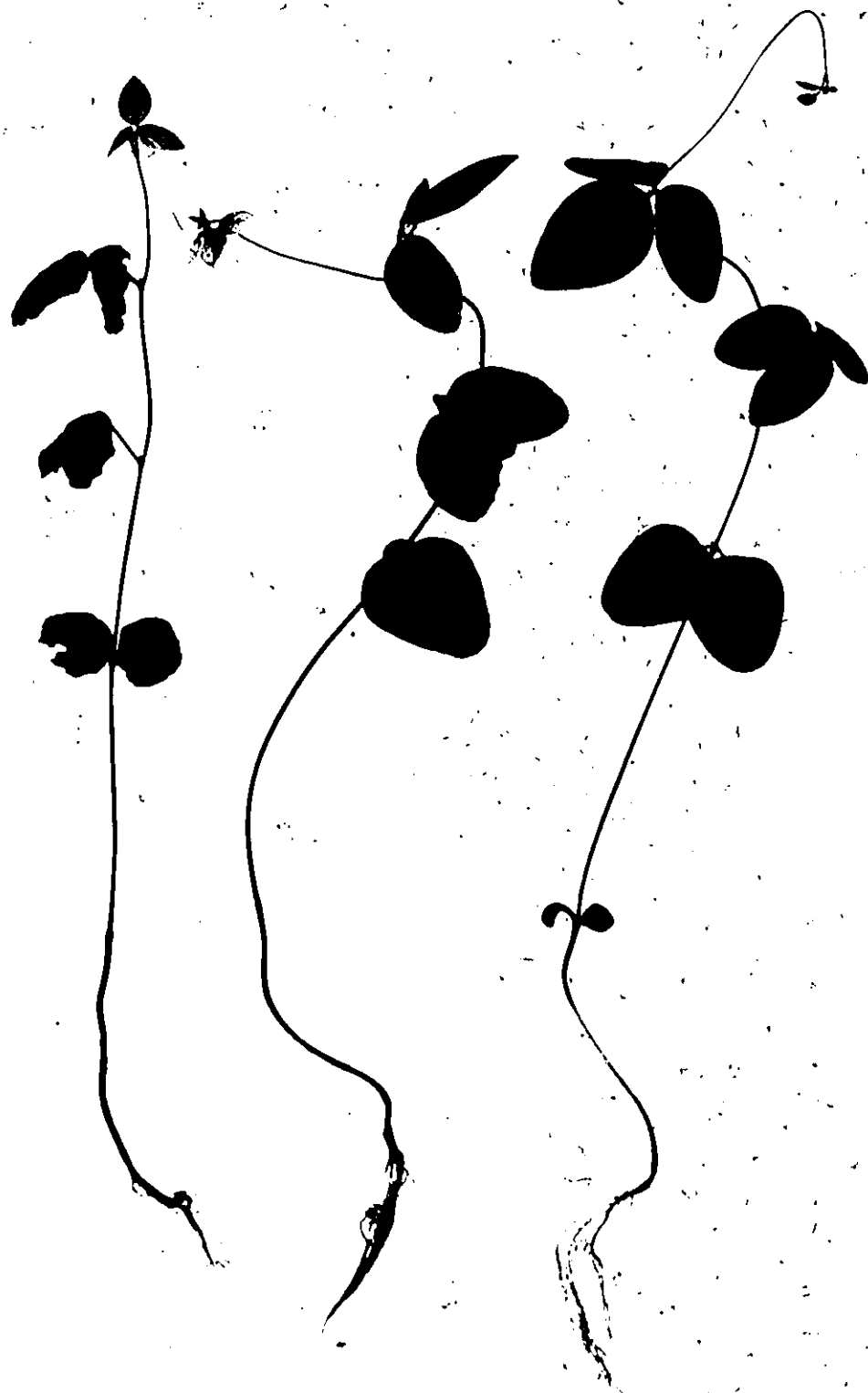


TABLE 11

Effect of uranium on total Chlorophyll, Chl_a and Chl_b from the leaves of four week old-soybean plants (as % of control)

Treatments μgU/mL	Total Chl	Chl _a	Chl _b
<u>Leaf 1 (oldest)</u>			
0.42	81 ± 5 a*	82 ± 5 a	77 ± 6 a
42.40	75 ± 13 a	76 ± 13 a	72 ± 15 a
<u>Leaf 2</u>			
0.42	76 ± 9 a	77 ± 9 a	74 ± 11 a
42.40	58 ± 17 b	59 ± 16 b	55 ± 20 b
<u>Leaf 3</u>			
0.42	93 ± 21 a	93 ± 18 a	96 ± 28 a
42.40	64 ± 12 b	68 ± 12 b	58 ± 10 b

* Means (n = 6) with standard deviation followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

B. URANIUM UPTAKE:

3.5.1. Uranium Accumulation in 6 or 7 d old Seedlings:

Uranium accumulation following germination indicated that uranium biosorption increased with increasing content of uranium in the medium (Figure 2). The total biosorption varied with the species in the order tomato > wheat > soybean on a dry weight basis.

Uranium analysis in the shoot-root axis and cotyledons of soybean, (Table 12) and shoot-root axis and endosperm of wheat (Table 13), when separately analysed, showed that uranium preferentially accumulated in the shoot-root axis. The ratio of uranium accumulated in these tissues as compared with that found in the cotyledons or endosperm was approximately 3:1. The accumulation of uranium in these tissues (separately) also increased with increasing content in the medium.

3.5.2. Application of Freundlich Model Equation to Uranium Biosorption:

When the biosorption data of the three plant species were graphically displayed (Figure 2), they resembled adsorption isotherm curves (Giles et al., 1960). Hence it was decided to fit the available biosorption data of all the three species with the most widely accepted biosorption isotherm model equation namely Freundlich's model equation, and individual Freundlich equations were calculated for each set of data.

FIGURE 2

Accumulation of uranium ($\mu\text{gU/g}$ dry wt.) in 6 d old soybean and wheat, and 7 d old tomato seedlings. Mean values of 6 replicates are shown with standard deviations.

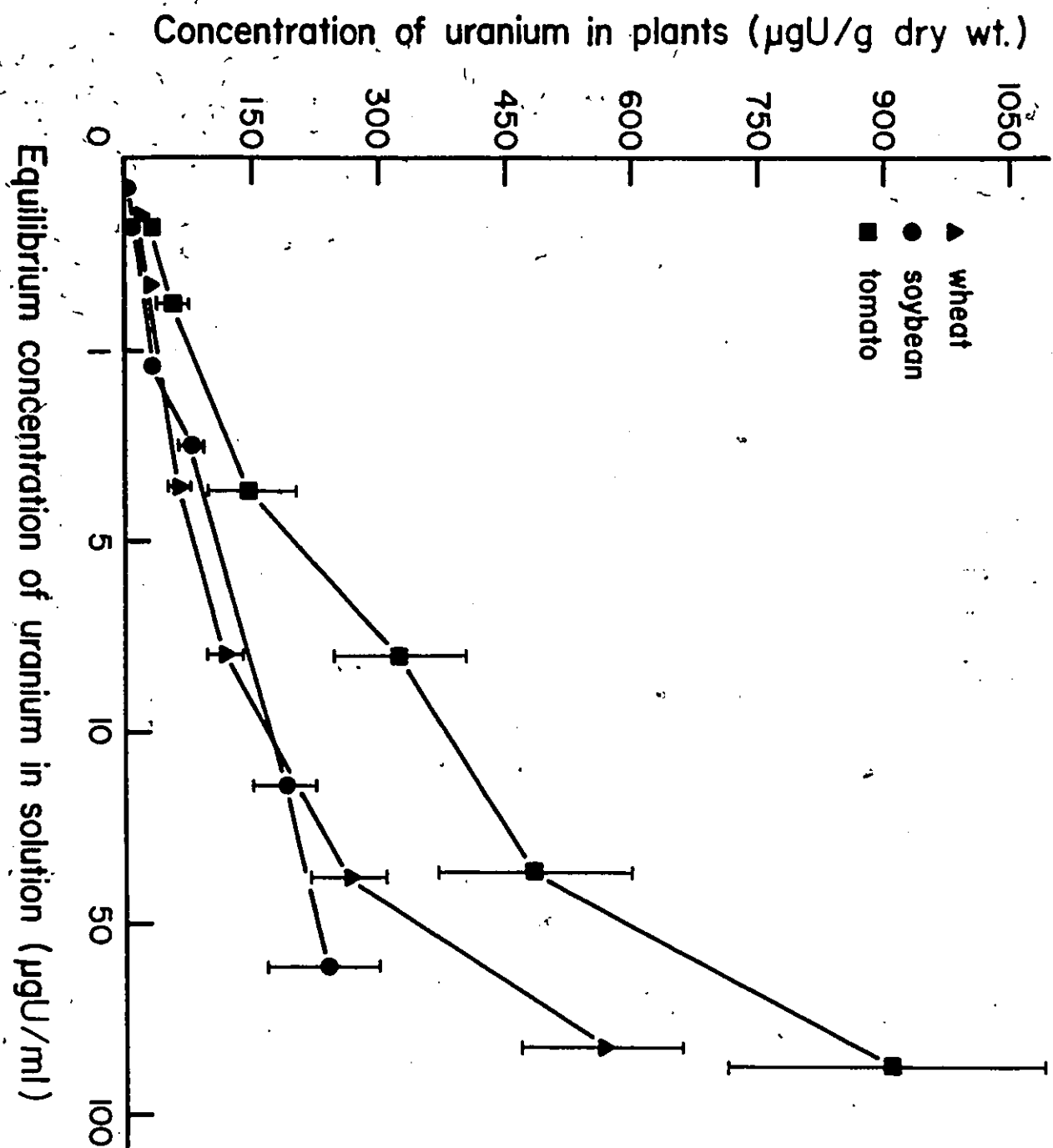


TABLE 12.

Accumulation of uranium ($\mu\text{gU/g}$ dry wt.) in the shoot-root axes,
and cotyledons of 6 d old soybean seedlings

Treatments $\mu\text{gU/mL}$	Shoot-root axes Mean $\pm$ S.D.	Cotyledons Mean $\pm$ S.D.
Control	1.80 $\pm$ 1.00	0.37 $\pm$ 0.10
0.42	3.19 $\pm$ 1.47	1.92 $\pm$ 0.26
0.84	4.44 $\pm$ 1.20	2.31 $\pm$ 0.24
4.24	20.70 $\pm$ 5.00	11.10 $\pm$ 2.00
8.48	45.20 $\pm$ 10.00	16.47 $\pm$ 2.00
42.40	141.00 $\pm$ 28.00	51.60 $\pm$ 13.00
84.80	175.00 $\pm$ 58.00	68.00 $\pm$ 17.00

* Means ($n = 6$) with standard deviations are presented.

TABLE 13

Accumulation of uranium ($\mu\text{gU/g dry wt.}$) in the shoot-root axes, and endosperm of 6 d old wheat seedlings

Treatments $\mu\text{gU/mL}$	Shoot-root axes Mean $\pm$ S.D.	Endosperm Mean $\pm$ S.D.
Control	2.65 $\pm$ 1.4	1.14 $\pm$ 0.5
0.42	10.30 $\pm$ 3.0	4.07 $\pm$ 1.5
0.84	16.30 $\pm$ 3.0	5.45 $\pm$ 1.7
4.24	48.00 $\pm$ 18.0	16.00 $\pm$ 3.1
8.48	84.00 $\pm$ 29.0	29.00 $\pm$ 10.0
42.40	181.00 $\pm$ 4.0	83.00 $\pm$ 34.0
84.80	438.00 $\pm$ 72.0	131.00 $\pm$ 34.0

* Means ($n = 6$) with standard deviations are presented.

The Freundlich equation is expressed as follows:

$$\frac{x}{m} = KC'^{1/n}$$

where $\frac{x}{m}$ is the amount of adsorbate (uranium) per unit amount of adsorbent (plant material), C' is the concentration of solute or adsorbate (uranium) upon achieving equilibrium, K and $1/n$ are the constants.

The Freundlich equation can be applied where a plot of $\log \frac{x}{m}$ versus $\log C'$ gives a straight line with an intercept of $\log K$ and slope $1/n$ (Choudhry, 1981). This was obtained for wheat, tomato and soybean (Figure 3).

Regression lines were drawn for the experimental data points which are expressed by equations;

$$\log \frac{x}{m} = 1.375 + 0.701 \log C' \text{ or } \frac{x}{m} = 23.739 C'^{0.701} \text{ (wheat),}$$

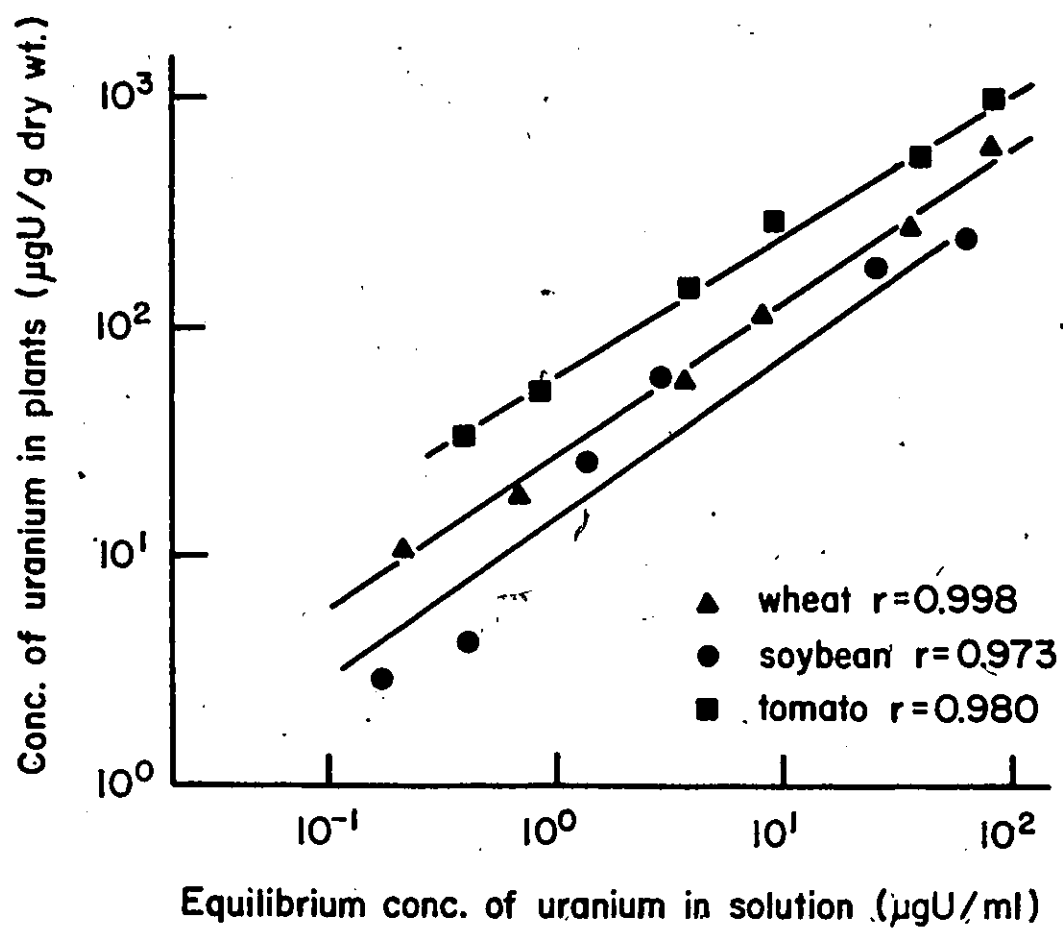
$$\log \frac{x}{m} = 1.166 + 0.777 \log C' \text{ or } \frac{x}{m} = 14.642 C'^{0.777} \text{ (soybean), and}$$

$$\log \frac{x}{m} = 1.795 + 0.607 \log C' \text{ or } \frac{x}{m} = 62.387 C'^{0.607} \text{ (tomato)}$$

To determine the fit of the Freundlich equation to the present experimental data, the coefficient of correlation was calculated. This was 0.998 for wheat, 0.973 for soybean and 0.980 for tomato. The significance of coefficient of correlation varied with the species in the order wheat > tomato > soybean. (See Appendix V for calculation).

FIGURE 3

Linearized biosorption isotherms for soybean, wheat and tomato. Data taken from Figure 2.



3.5.3 Uranium Accumulation in 4 wk old Soybean Plants:

In 4 wk old soybean plants, as in the case of 6 or 7 d seedlings (Section 3.5.1.), uranium biosorption increased with an increase in the concentration of uranium in the medium (Table 14). The accumulated uranium was preferentially located in the root system. The ratio of uranium in the roots:shoots was 10:1 at a concentration of 42.0 $\mu\text{gU/mL}$ uranium in the medium.

3.5.4. Influence of Additional Calcium on Uranium Uptake:

Following exposure to 84.80 $\mu\text{gU/mL}$ for a 10 d experimental period, an increase in the content of calcium from 80 to 180 $\mu\text{gU/mL}$ in the medium resulted in a significant ($P < 0.1$) decrease of 20% and 24% uranium uptake by wheat seedling roots and shoots respectively (Table 15). Uranium taken up by the seedlings was preferentially accumulated in the root system. The ratio of uranium in the roots:shoots was approximately 12:1 in both the sets.

3.5.5. Influence of pH on Uranium Uptake:

The uptake of uranium was markedly influenced by the pH of the ambient solution following a 10 d exposure period to 8.48 $\mu\text{gU/mL}$ (Figure 4). The maximum uranium uptake (516 ± 116 and $417 \pm 189 \mu\text{gU/g dry wt.}$), was obtained at pH 5.0 and pH 6.0. There was no significant difference

TABLE 14

Accumulation of uranium in the shoot and root system of four week old soybean plants ($\mu\text{gU/g}$ dry wt. $\pm$ S.D.)

Treatments $\mu\text{gU/mL}$	Shoot Mean $\pm$ S.D.	Root Mean $\pm$ S.D.
Control	0.62 $\pm$ 0.03	4.09 $\pm$ 0.21
0.42	1.37 $\pm$ 0.07	57.01 $\pm$ 2.90
42.40	91.51 $\pm$ 4.50	938.10 $\pm$ 22.60

* Means (n = 6) with standard deviations are presented.

TABLE 15

The influence of calcium on the uptake of uranium in wheat seedlings
10 d post treatment

Treatments $\mu\text{gU/mL}$	$\mu\text{gU/g dry wt.} \pm \text{S.D.}$	
	Shoot	Root
84.80 + 100 $\mu\text{gCa}^{2+}/\text{mL}$	84 $\pm$ 9 a	965 $\pm$ 83 a
84.80	105 $\pm$ 30 b	1279 $\pm$ 358 b

Means ($n = 6$) with standard deviation followed by the same letter are not significantly different by Student t-test at $P < 0.1$ level.

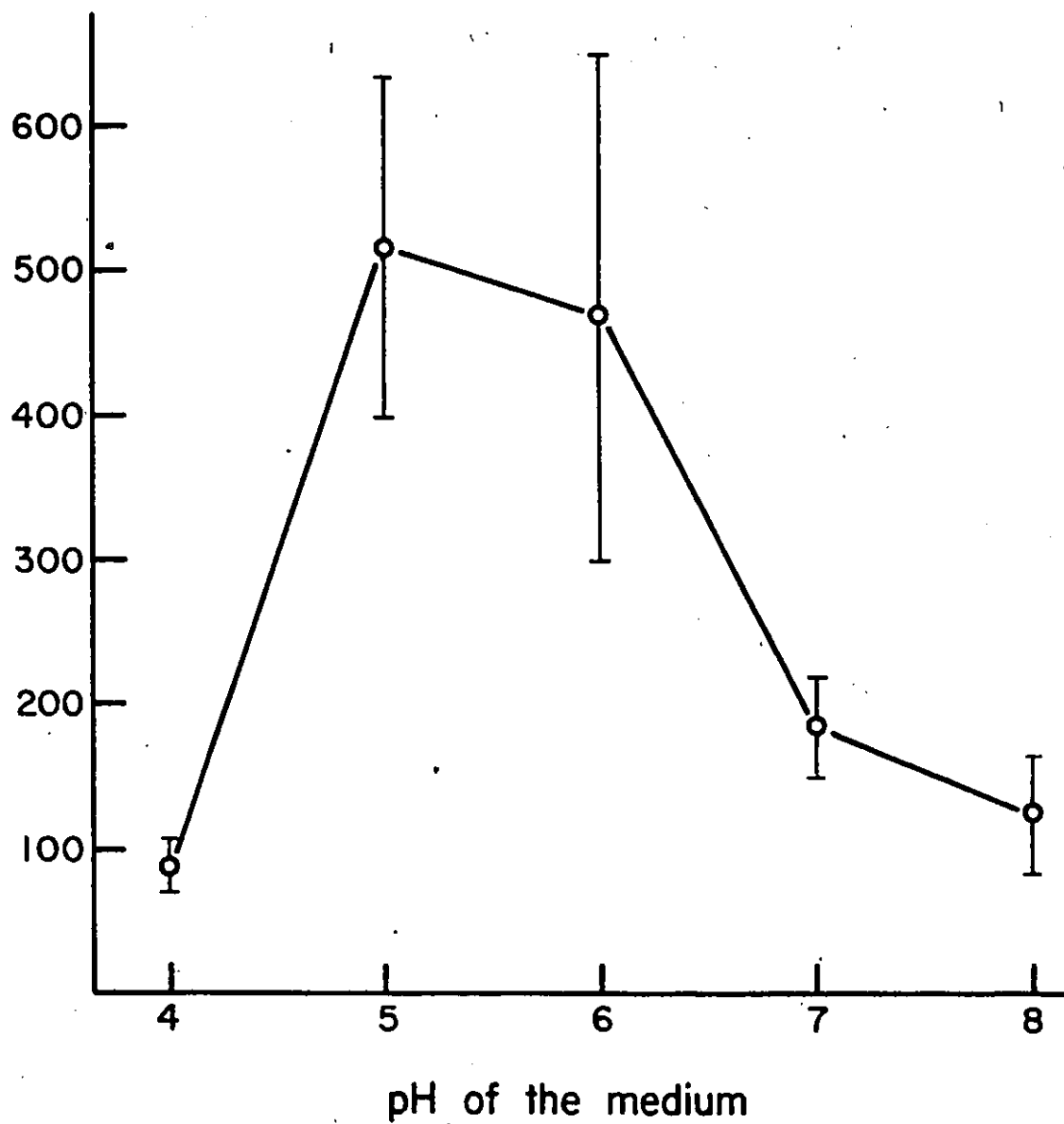
in uranium uptake between pH 5.0 and pH 6.0. Comparatively less uranium was taken up by wheat seedlings at pH 4.0 ($86 \pm 14 \mu\text{gU/g}$), pH 7.0 ($185 \pm 30 \mu\text{gU/g}$) and pH 8.0 ($125 \pm 45 \mu\text{gU/g}$) than at pH 5.0 and pH 6.0.

2

FIGURE 4

Effect of pH on the uptake of uranium by 14 d old wheat seedlings exposed to 8.48 $\mu\text{gU/mL}$. Means of 6 replicates are shown with standard deviations.

Concentration of uranium in the seedlings ($\mu\text{gU/g dry wt.}$)



3.5.6. Spontaneous Changes in the pH of the Media:

The accumulation of uranium was accompanied by an increase in the pH of the germination media (Table 16). The pH of the media was measured on day 0 and after 6 or 7 d germination. In tomato, the pH of the bathing medium of control set did not change significantly from the initial pH 5.4. However, uranium treated sets of tomato showed a significant increase in pH after 7 d germination. A significant increase of 1.66 pH units was obtained in the medium by uranium treatments of 4.24 and 8.48 $\mu\text{gU/mL}$ respectively. An even greater increase of 2.3 and 2.5 pH units respectively was brought about by exposure of the tomato seedlings to the two higher uranium treatments of 42.40 and 84.80 $\mu\text{gU/mL}$.

In the media containing soybean and wheat seeds, a pH increase of approximately 2.0 pH units was noted in both the control and uranium treated sets during the course of germination. In the treated sets alone, there was a trend towards increasing pH with increasing uranium content of the media. In the sets containing 84.80 $\mu\text{gU/mL}$ an increase of 0.6 pH units was obtained in the presence of soybean seeds and an increase of 0.9 pH unit in presence of wheat grains.

TABLE 16
Effect of uranium on the pH of the germination media
before and after germination

Treatments $\mu\text{gU/ml}$	Before germination	After germination
<u>Tomato</u>		
		After 7 days
Control	5.4 $\pm$ 0	5.43 $\pm$ 0.31
4.24	5.4 $\pm$ 0	7.06 $\pm$ 0.08
8.48	5.4 $\pm$ 0	7.07 $\pm$ 0.08
42.40	5.4 $\pm$ 0	7.71 $\pm$ 0.08
84.80	5.4 $\pm$ 0	7.86 $\pm$ 0.03
<u>Soybean</u>		
		After 6 days
Control	5.6 $\pm$ 0	7.58 $\pm$ 0.16
4.24	5.6 $\pm$ 0	7.54 $\pm$ 0.12
8.48	5.6 $\pm$ 0	7.71 $\pm$ 0.13
42.40	5.6 $\pm$ 0	7.90 $\pm$ 0.04
84.80	5.6 $\pm$ 0	8.10 $\pm$ 0.06
<u>Wheat</u>		
		After 6 days
Control	5.8 $\pm$ 0	7.36 $\pm$ 0.15
4.24	5.8 $\pm$ 0	7.85 $\pm$ 0.03
8.48	5.8 $\pm$ 0	7.95 $\pm$ 0.04
42.40	5.8 $\pm$ 0	8.20 $\pm$ 0.12
84.80	5.8 $\pm$ 0	8.25 $\pm$ 0.22

Means (n = 10) with standard deviations

C. ION FLUX AND MEMBRANE PERMEABILITY

3.6.1. Electrical Conductivity:

It was noted that the addition of uranium oxide to the Hoagland's nutrient solution increased the conductivity of the medium in the uranium treated sets (Figure 5). A significant increase in the conductivity was obtained at the two higher uranium treatment levels compared with control in soybean and tomato sets (Figures 5 A, B). Whereas in wheat, significant changes in the conductivity were obtained for all levels of treatments (Figure 5 C).

In each of the control and uranium treated sets, no significant subsequent changes were observed during the experimental period (Figures 5A, B, C). However, when the conductivity values were converted to percentage of control, a trend towards an increase in conductivity of the media was noted in the sets containing tomato and wheat at the two higher treatment levels (Figures 6 B, C).

FIGURE 5

- 5A. Effects of uranium on the electrolyte leakage into the germination medium of soybean seeds and seedlings during the course of germination. A plot of electrolyte leakage (mS) vs. time (days). Means of 9 replicates are shown with standard deviations.
- 5B. Effects of uranium on the electrolyte leakage into the germination medium of tomato seeds and seedlings during the course of germination. A plot of electrolyte leakage (mS) vs. time (days). Means of 9 replicates are shown with standard deviations.
- 5C. Effects of uranium on the electrolyte leakage into the germination medium of wheat seeds and seedlings during the course of germination & early growth. A plot of electrolyte leakage (mS) vs. time (days). Means of 6 replicates are shown with standard deviations.

FIGURE 5A.

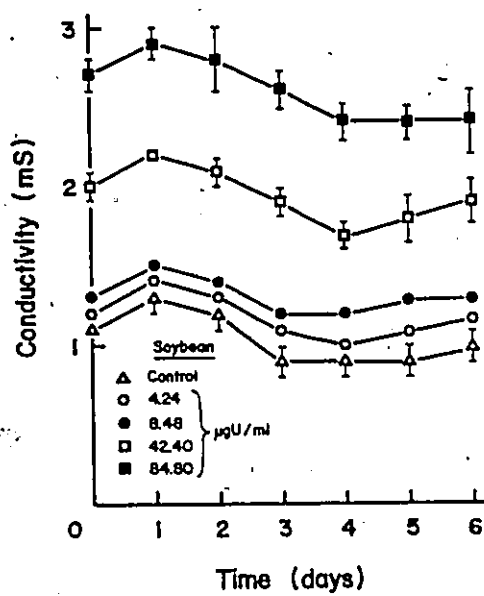


FIGURE 5B.

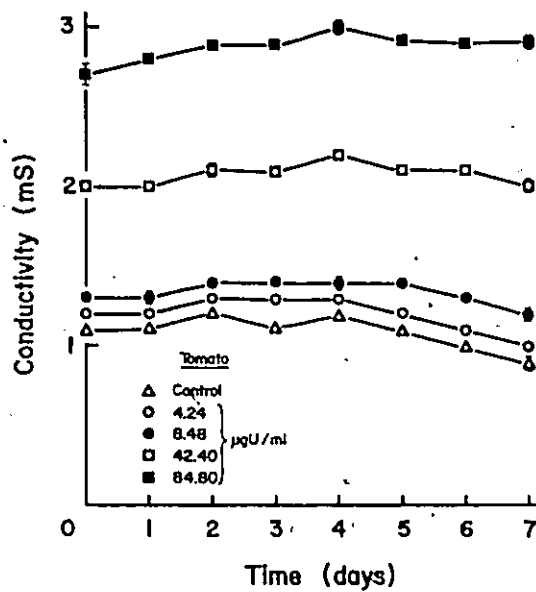


FIGURE 5C.

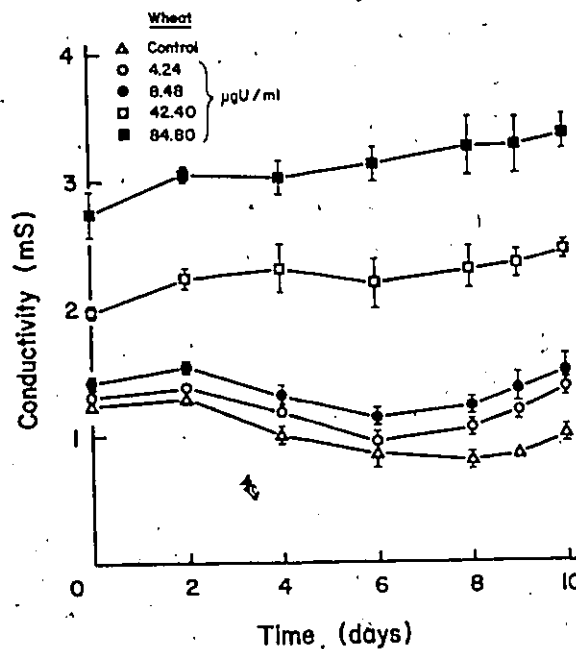


FIGURE 6

- 6A. Effect of uranium on the electrolyte leakage into the germination medium of soybean seeds and seedlings during the course of germination. Electrolyte leakage is represented as % of control vs. time (days). Data taken from Figure 5A.
- 6B. Effect of uranium on the electrolyte leakage into the germination medium of tomato seeds and seedlings during the course of germination. Electrolyte leakage is represented as % of control vs. time (days). Data taken from Figure 5B.
- 6C. Effect of uranium on the electrolyte leakage into the germination medium of wheat seeds and seedlings during the course of germination & early growth. Electrolyte leakage is represented as % of control vs. time (days). Data taken from Figure 5C.

FIGURE 6A.

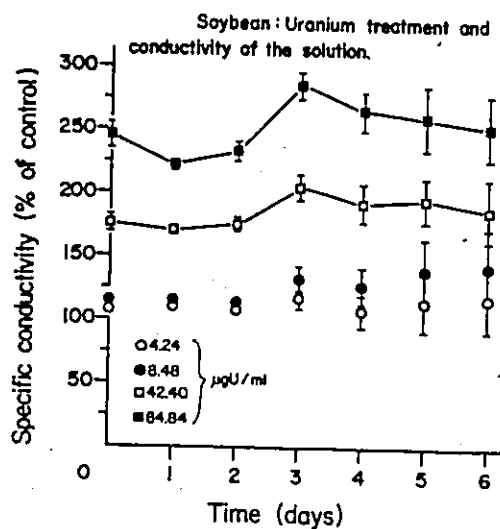


FIGURE 6B.

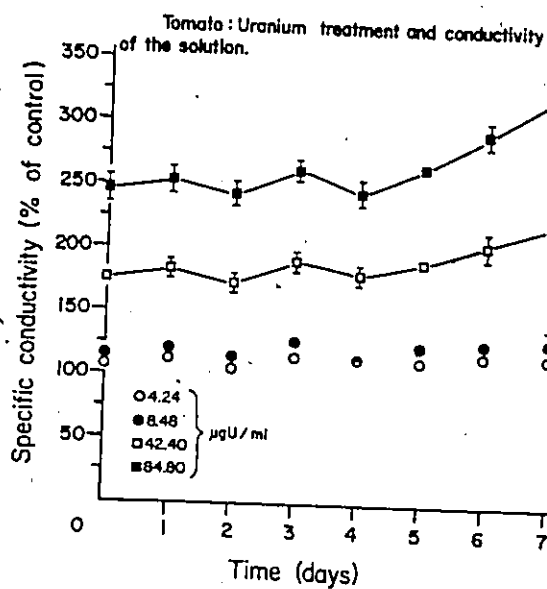
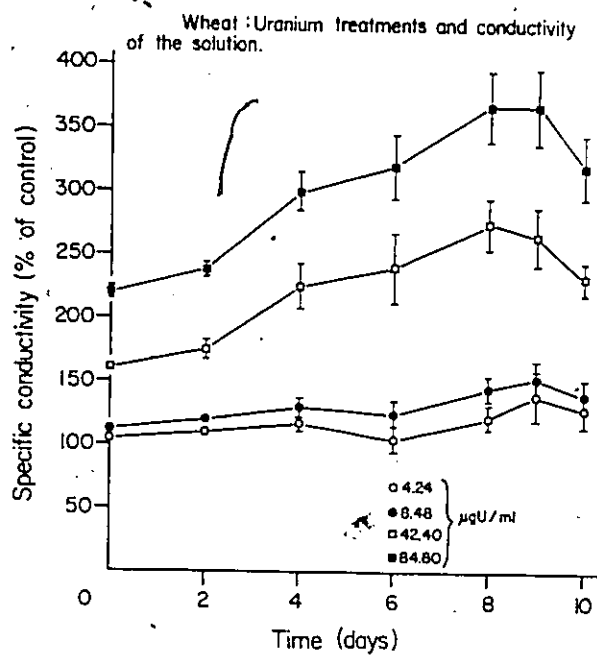


FIGURE 6C.



3.6.2. Potassium Flux:

During the course of germination, significant specific differences were observed in the flux of potassium. In soybean (Figure 8), the imbibitional leakage and the subsequent influx of K^+ was similar in the control and all the uranium treated sets 0 to 4 d germination. During the later part of the germination period (4 to 6 d) there was no significant influx or efflux of K^+ in the control sets, whereas significant efflux was obtained in all the treatment sets. The two lower levels of uranium treatment, namely 4.24 and 8.48 $\mu\text{gU/mL}$ caused a 0.3 and 0.5 fold increase in K^+ in the media respectively during 5 to 6 d germination. This represents a 30% and 50% efflux of K^+ during that period. (Figure 7A). In the two higher treatment levels (42.40 and 84.80 $\mu\text{gU/mL}$ during the 4 to 6 d germination) a 0.7 and 0.9 fold increase in K^+ was obtained in the media in which the seedlings were immersed compared with the control sets. This indicates that a 70% and 90% efflux of K^+ took place at these two higher treatment levels (Figure 7B).

In tomato (Figure 8), the K^+ in the germinating media of control and all the uranium treatment sets showed a marginal fluctuation during the early period of 0 to 4 d germination. No significant differences between the control and uranium treatments were observed during this period. From 4 to 7 d, there was a rapid decrease in K^+ in the control sets indicating the influx of K^+ . The decrease in K^+ in the media containing 4.24 and 8.48 $\mu\text{gU/mL}$ was 0.14 and 0.21 fold less than that obtained in the control sets. This represents a 14% and 21% decrease in

FIGURE 7

- 7A. Effect of uranium on K^+ flux in soybean determined by measuring the K^+ ($\mu\text{g}/\text{mL}$) in the germination medium. A plot of K^+ ($\mu\text{g}/\text{mL}$) in the germination medium of control, treated with 4.24, and 8.48 $\mu\text{gU}/\text{mL}$ vs. time (days). Means of 9 replicates are shown with standard deviations.
- 7B. A plot of K^+ ($\mu\text{g}/\text{mL}$) in the germination medium of control, treated with 42.40, and 84.80 $\mu\text{gU}/\text{mL}$ vs. time (days). Means of 9 replicates are shown with standard deviations.

FIGURE 7A.

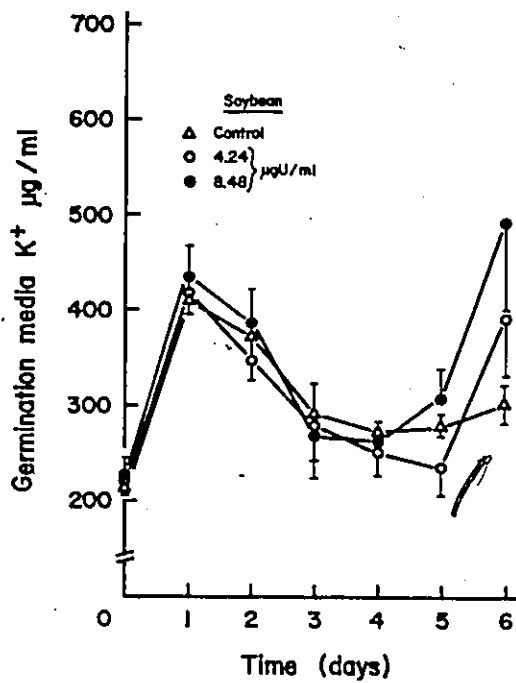
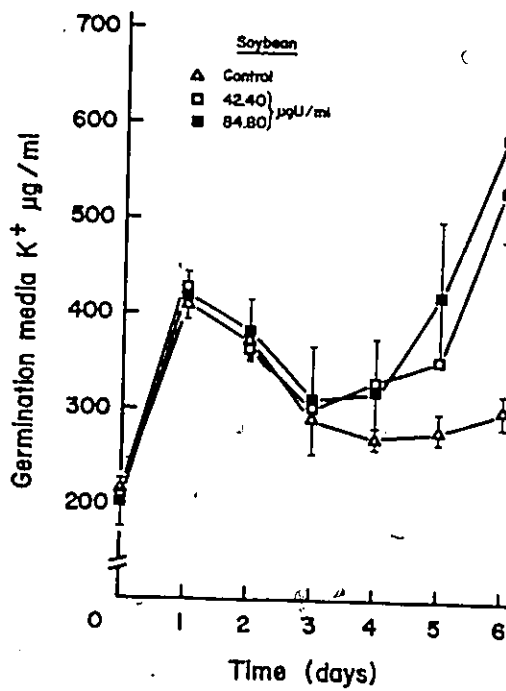


FIGURE 7 B.



K⁺ influx (Figure 8A). By contrast, the K⁺ flux in the set exposed to 42.40 $\mu\text{gU/mL}$ (Figure 8B) did not change significantly from the initial level during the 4 to 7 d germination period. During the same 4 to 7 d germination period, the sets exposed to 84.80 $\mu\text{gU/mL}$ exhibited a 0.2 fold increase in K⁺ in the media over the initial level representing a 20% net efflux of K⁺ into the germination media at the highest uranium treatment level.

In wheat (Figure 9), the K⁺ level in the medium was similar in the control and all the uranium treated sets during the first 2 d germination. Thereafter, there was a rapid decrease in K⁺ in the medium of the control set indicating an influx into the seeds and seedlings, and an insignificant increase between 9 and 10 d. The K⁺ level in the media of the uranium treated sets exposed to 4.24 and 8.48 $\mu\text{gU/mL}$ decreased 4 to 6 d germination and increased thereafter. The increase was 0.15 and 0.21 fold respectively between 6 and 10 d at these two lower levels. This implies a 15 and 21% efflux of K⁺ respectively at these levels of uranium treatment (Figure 9A). By contrast, the two higher uranium treatments 42.40 and 84.80 $\mu\text{gU/mL}$ significantly increased the K⁺ content of the media from the initial level. This increase was 0.56 and 0.69 fold respectively representing a 56% and 69% efflux of K⁺ by uranium compared with the control sets (Figure 9B). This very clearly indicates that there was both an inhibition of K⁺ influx and a stimulation of K⁺ efflux evoked by the uranium treatments.

FIGURE 8

- 8A. Effect of uranium on K^+ flux in tomato determined by measuring the K^+ ($\mu\text{g/mL}$) in the germination medium. A plot of K^+ ($\mu\text{g/mL}$) in the germination medium of control, treated with 4.24, and 8.48 $\mu\text{gU/mL}$ vs. time (days). Means of 9 replicates are shown with standard deviations.
- 8B. A plot of K^+ ($\mu\text{g/mL}$) in the germination medium of control, treated with 42.40, and 84.80 $\mu\text{gU/mL}$ vs. time (days). Means of 9 replicates are shown with standard deviations.

FIGURE 8A.

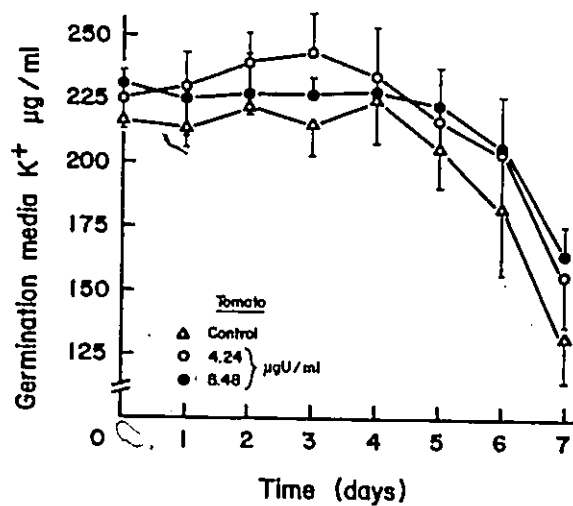
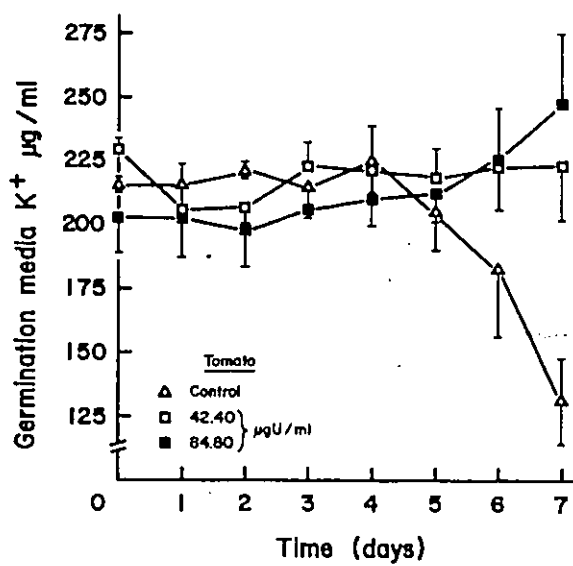


FIGURE 8B.



3

FIGURE 9

- 9A. Effect of uranium on K^+ flux in wheat determined by measuring the K^+ ($\mu\text{g/mL}$) in the germination medium. A plot of K^+ ($\mu\text{g/mL}$) in the germination medium of control, treated with 4.24, and 8.48 $\mu\text{gU/mL}$ vs. time (days). Means of 9 replicates are shown with standard deviations.
- 9B. A plot of K^+ ($\mu\text{g/mL}$) in the germination medium of control, treated with 42.40, and 84.80 $\mu\text{gU/mL}$ vs. time (days). Means of 6 replicates are shown with standard deviations.

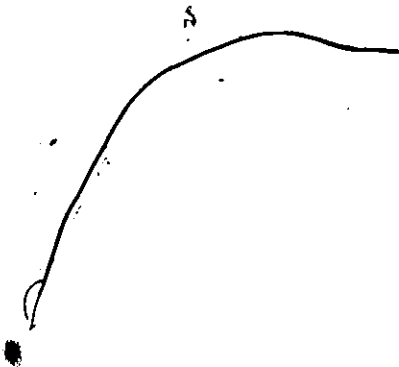


FIGURE 9A.

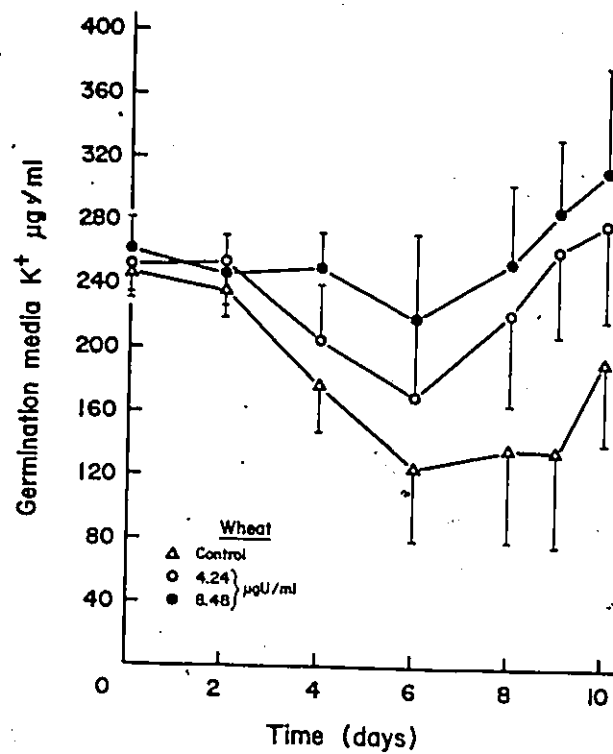
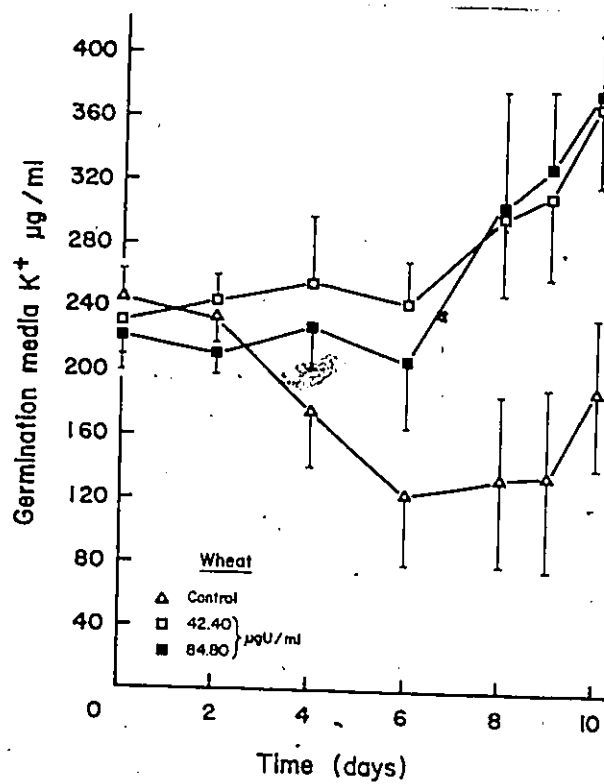


FIGURE 9B.



3.7. K⁺, Na⁺ and Mg²⁺ Accumulation in the Seedlings:

The accumulation of potassium, sodium and magnesium by the wheat seedlings of control and uranium treated sets following 10 d growth are shown in Tables 17 - 19. The uranium treatments resulted in the selective inhibition of K⁺ and Mg²⁺ accumulation and a stimulated accumulation of Na⁺.

In wheat roots,, the accumulation of K⁺ was decreased by 45% following exposure to 42.40 and 84.80 $\mu\text{gU/mL}$ (Table 17). This was paralleled by a highly significant accumulation of 477% and 563% Na⁺ representing an increase over the control sets (Table 18).

The ratio of K⁺:Na⁺ in the control sets was as 3.5:1. This showed a clear selectivity of K⁺ and Na⁺ accumulation in the control sets. By contrast, in the uranium treated sets the ratio was reversed and the ratio of 1:3 (K⁺:Na⁺) was obtained even when the ratio of K⁺:Na⁺ in the bathing media was 1:1 in both the control and treated sets.

During the same time period, the accumulation of Mg²⁺ in the wheat seedlings decreased by 35 to 45% following exposure to 42.40 and 84.80 $\mu\text{gU/mL}$ respectively compared to the control seedlings (Table 19).

TABLE 17

Effect of uranium on potassium accumulation in the roots
of wheat seedlings 10 d post treatment

Treatments $\mu\text{gU/mL}$	mM K^+ /g dry wt. Means $\pm$ S.D.	% of control
Control	321 $\pm$ 45 a*	
42.40	181 $\pm$ 57 b	56 $\pm$ 17
84.80	180 $\pm$ 45 b	55 $\pm$ 13

TABLE 18

Effect of uranium on sodium accumulation in the roots
of wheat seedlings 10 d post treatment

Treatments $\mu\text{gU/mL}$	mM Na^+ /g dry wt. Means $\pm$ S.D.	% of control
Control	92 $\pm$ 24 a*	
42.40	440 $\pm$ 29 b	477 $\pm$ 32
84.80	518 $\pm$ 46 b	563 $\pm$ 49

* Means (n = 6) with standard deviation followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).

TABLE 19

Effect of uranium on magnesium accumulation in the roots
of wheat seedlings 10 d post treatment

Treatments $\mu\text{gU/mL}$	mM Mg^{2+} /g dry wt. Means $\pm$ S.D.	% of control
Control	154.99 $\pm$ 38.79 a*	
42.40	101.44 $\pm$ 12.71 b	65 $\pm$ 08
84.80	83.35 $\pm$ 08.03 b	54 $\pm$ 05

* Means ($n = 6$) with standard deviation followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).

3.8. Total Root ATP Content:

Total ATP content of the wheat roots was determined on days 2, 4, 6, 8 and 10 during the course of germination and early growth (Figure 10). During this period, the total ATP content of the wheat roots was depressed by 40 to 75% by uranium treatments $\geq 4.24 \mu\text{gU/mL}$. Between 4 and 10 d early growth, a 20 to 40% decrease in ATP content was recorded following exposure to 4.24 and 8.48 $\mu\text{gU/mL}$, respectively. The two higher concentrations, namely, 42.40 and 84.80 $\mu\text{gU/mL}$ brought about a 30 to 75% decrease respectively in the ATP content during a comparable growth period.

3.9. Scanning Electron Microscopy (SEM):

Scanning electron micrographs of untreated roots of wheat and roots exposed to 84.80 $\mu\text{gU/mL}$ for a period of 10 d are shown in Figures 11-13. Exposure to 84.80 $\mu\text{gU/mL}$ caused a reduction in the growth of the root hairs and disruption of the root cap (Figures 11B, 12B). There is no evidence (Figure 12) of uranium deposition on the exterior surface of the treated root tip. In addition, the lateral roots in the uranium treated sets were reduced (Figure 13B) compared to the control (Figure 13A).

FIGURE 10

Effect of uranium on the total root ATP content of wheat. Total ATP content is represented as % of control vs. time (days). Means of 4 replicates with standard deviations are shown.

Uranium treatments and total ATP content of wheat roots

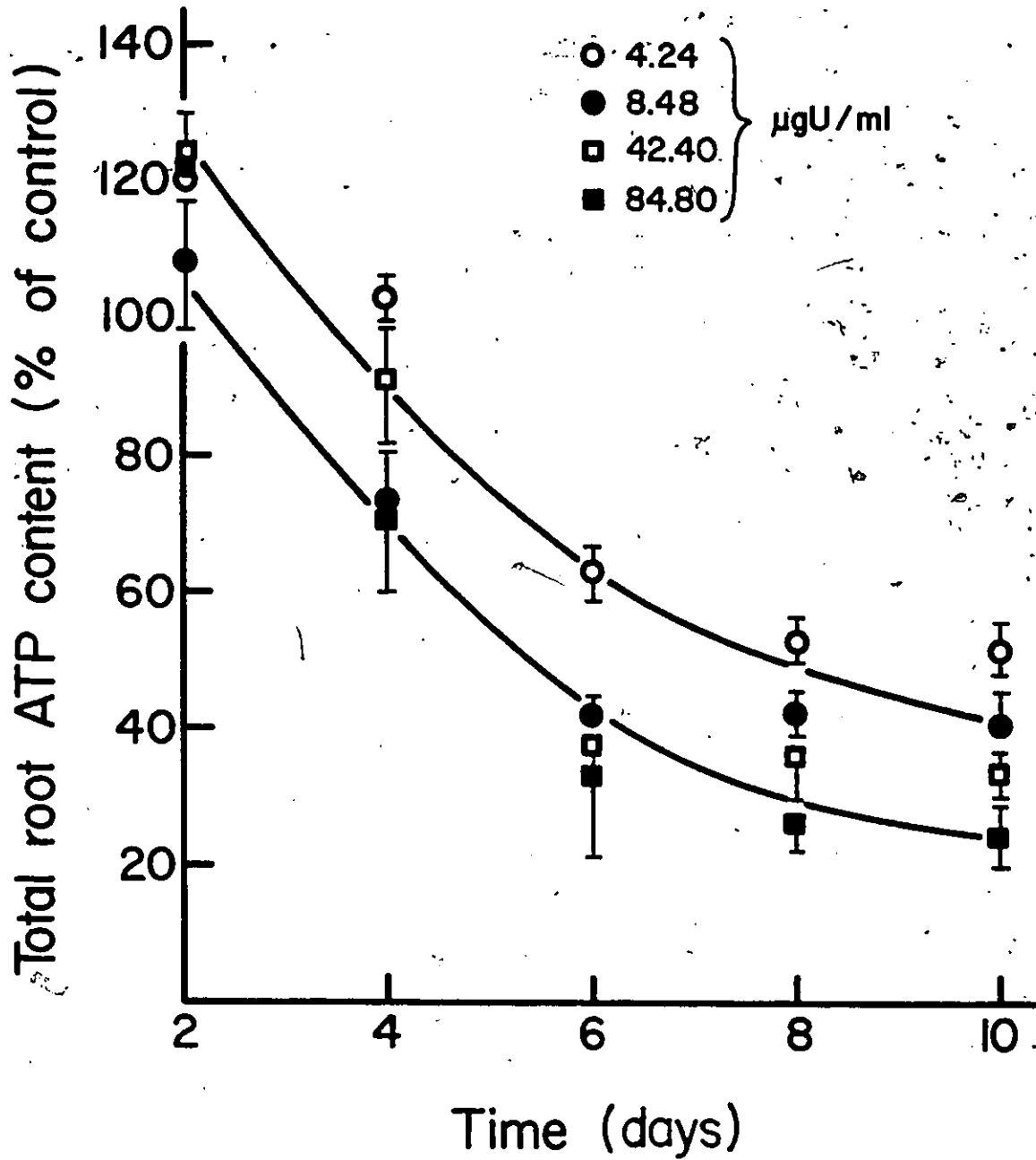


FIGURE 11A & 11B

Scanning electron micrographs of wheat roots 10 d post-treatment.
A. Control roots showing normal root hairs 75X. B. Root treated with
84.80 μ gU/mL showing shortened root hairs 75X.
Figure legend: Root hairs (RH), Root cap (RC).

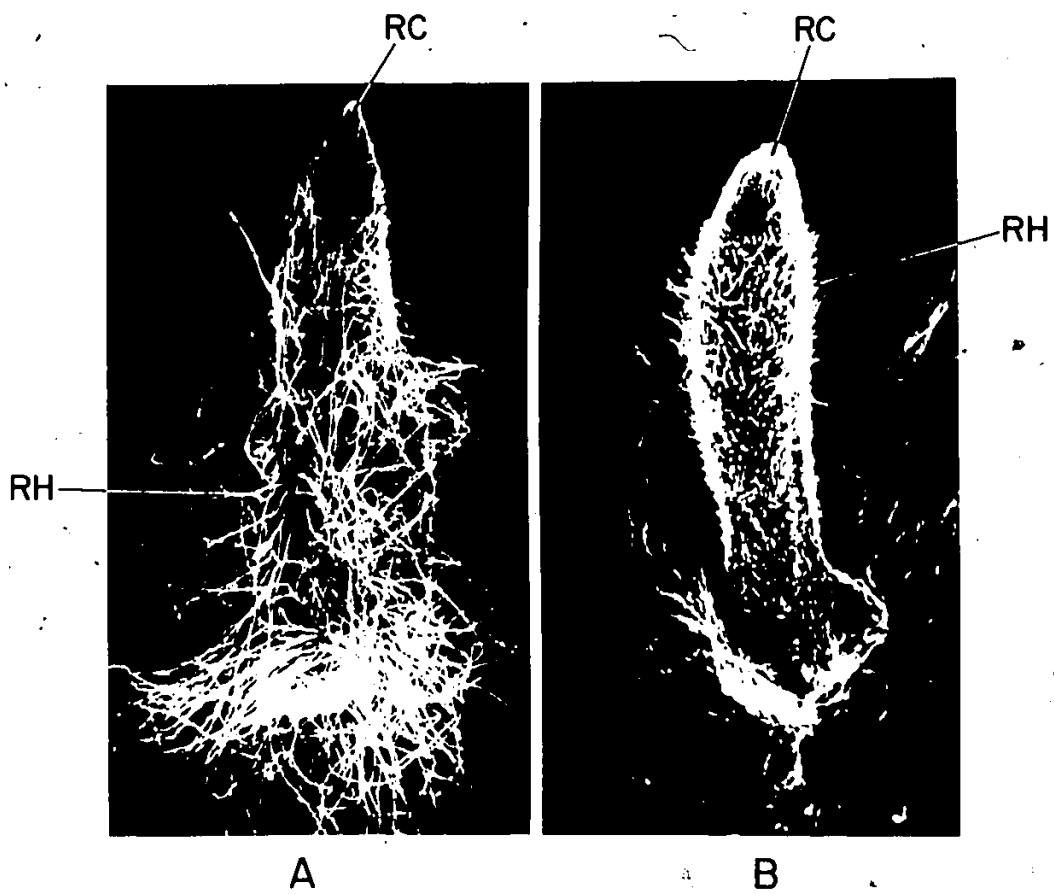


FIGURE 12A & 12B

Scanning electron micrographs of wheat roots 10 d post-treatment.
A. Control roots showing normal root cap 275X. B. Root treated with
84.80 $\mu\text{g}/\text{mL}$ showing disrupted root cap 275X.
Figure legend: Root hairs (RH), Root cap (RC).

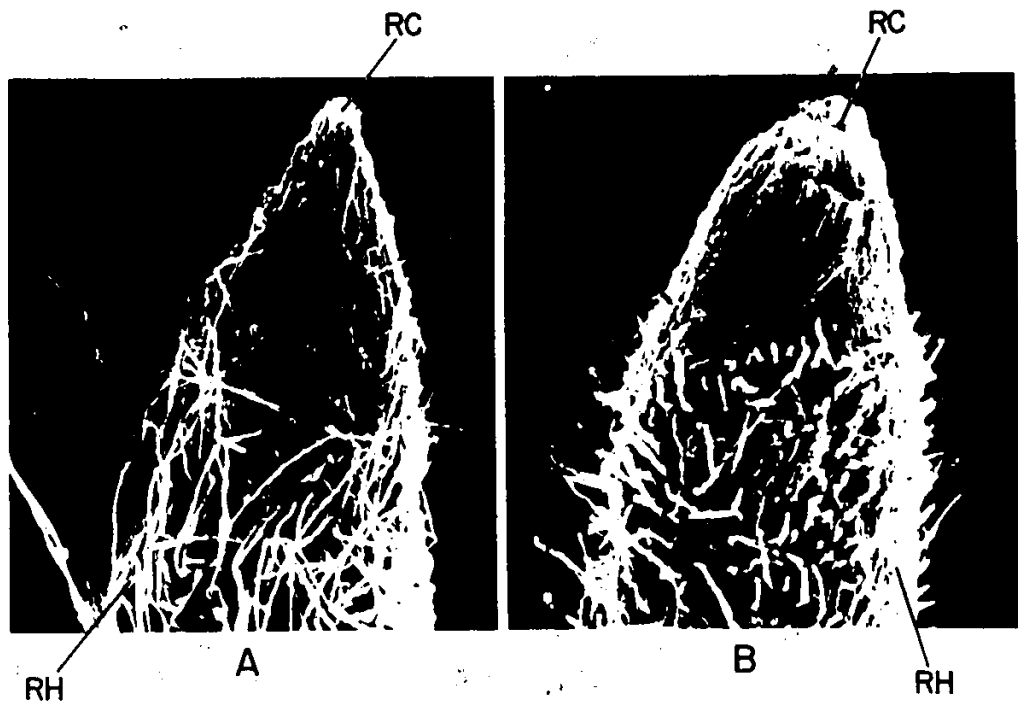
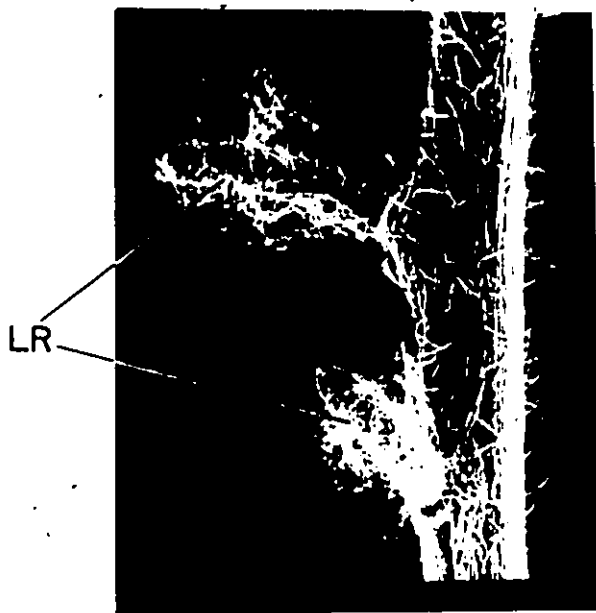


FIGURE 13A & 13B

Scanning electron micrographs of wheat roots 10 d post-treatment.
A. Control roots showing normal lateral root growth 100X. B. Root
treated with 84.80 $\mu\text{gU/mL}$ showing reduced lateral root growth 100X.
Figure legend: Lateral root (LR)



A



B

3.10. Transmission Electron Microscopy (TEM):

Conspicuous ultrastructural changes were observed in the meristematic cells of root tips exposed to 84.80 $\mu\text{gU/mL}$ uranium (Figures 14-17).

Cells in the centre of the meristematic zone of the uranium treated roots were characterized by cell walls with relatively high electron density (Figure 14B) compared to the untreated cells (Figure 14A). Invaginations of the plasmalemma were evident in the uranium treated cells (Figures 14A, 15B, 15C), and these were filled with vesicles of varying sizes. Additionally, relatively small electron dense particles were observed within these invaginations (Figure 14A, 15B, 15C).

The ultrastructural abnormalities of the plasmalemma were also observed in the peripheral cells of uranium treated wheat root tip meristem. These cells also showed relatively heavily labelled cell walls and plasmalemma dilations (Figure 16B, 16C, 17B). These dilations appeared to form invaginations and pinocytotic vesicles which were smaller and more numerous than was the case for cells in the centre of the meristematic zone (Figure 15B and 15C). By contrast, the subcellular organelles such as nuclei, mitochondria and proplastids appeared unaffected by the uranium treatment (Figures 14B, 15B, 15C, 16B).

FIGURE 14A & 14B

Transmission electron micrographs of longitudinal section of wheat root tip meristem. A. Control cells showing normal cell wall, plasmalemma and subcellular organelles 11250X. B. Cells exposed to 84.80 μ gU/mL showing cells walls with relatively high electron density and plasmalemma invaginations: 11250X.

Figure legend: Cell wall (CW), Plasmalemma (P), Nucleus (N), Nucleolus (Nu), Mitochondria (M), Proplastid (Pp), Vacuole (V), Plasmalemma invagination (PI).



FIGURE 15A & 15B

Transmission electron micrographs of longitudinal section of wheat root tip meristem. A. Control cell showing normal cell wall and plasmalemma 25000X. B. Cells exposed to 84.80 $\mu\text{gU/mL}$ showing cell wall with high electron density and plasmalemma invaginations filled with vesicles 25000X.

Figure legend: Cell wall (CW), Plasmalemma (P), Nucleus (N), Nucleolus (Nu), Mitochondria (M), Proplastid (Pp), Plasmalemma invagination (PI), Vesicle (Vs).

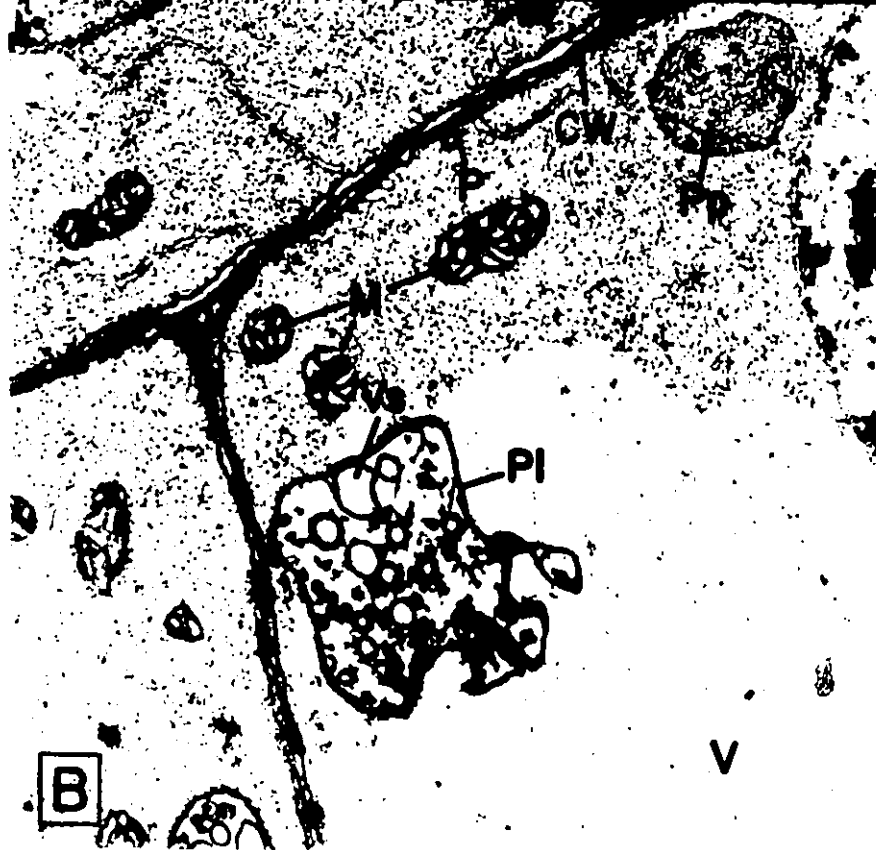
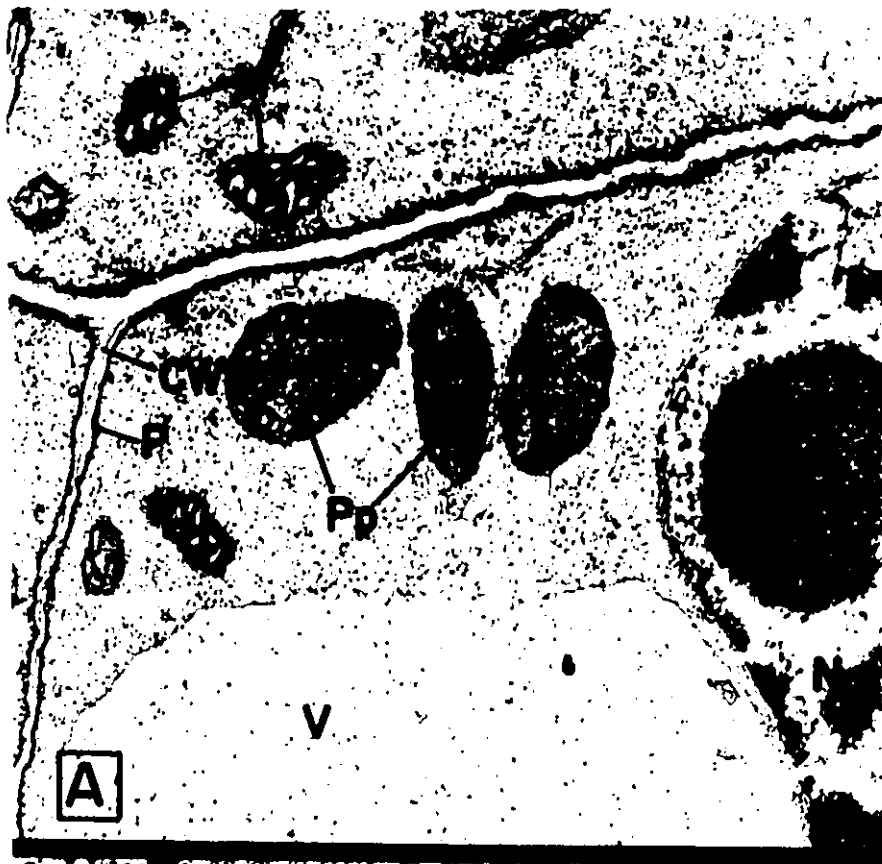


FIGURE 15C

/ A portion of a cell exposed to 84.80 μ gU/mL showing cell wall with heavy labelling and plasmalemma invagination filled with vesicles of varying size 37000X.

Figure legend: Cell wall (CW), Plasmalemma (P), Mitochondria (M), Plasmalemma invagination (PI), Vacuole (V), Vesicles (Vs). -



FIGURE 16A & 16B

Transmission electron micrographs of longitudinal section of wheat root-tip meristem (peripheral cells). A. Control cell showing normal cell wall, plasmalemma and subcellular organelles 25000X. B. Cell exposed to 84.80 $\mu\text{gU/mL}$ showing heavily labelled cell wall and plasmalemma dilations 25000X.

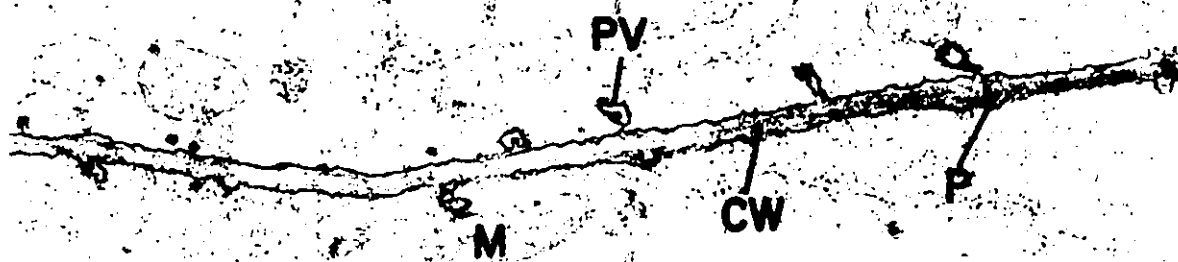
Figure legend: Cell wall (CW), Plasmalemma (P), Mitochondria (M), Proplastid (Pp), Vacuole (V), Plasmalemma dilation (PD).



FIGURE 16C

Transmission electron micrograph of longitudinal section of wheat root-tip meristem (peripheral cells) exposed to 84.80 μ gU/mL. A portion of the cell showing heavily labelled cell wall and pinocytotic vesicles 25000X.

Figure legend: Cell wall (CW), Plasmalemma (P), Mitochondrion (M), and Pinocytotic vesicle (PV).

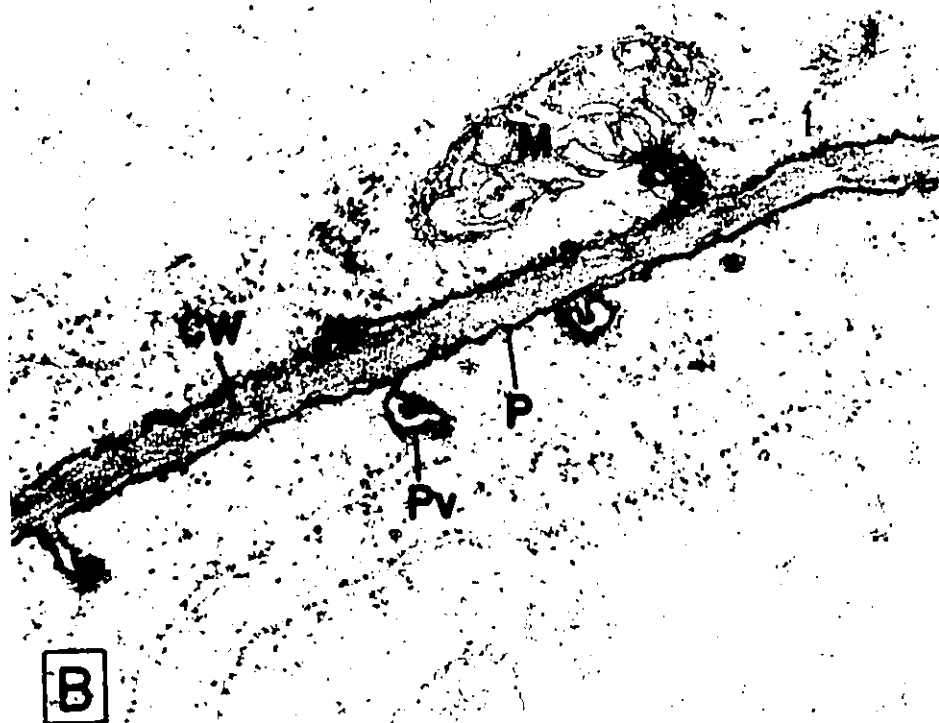


C

FIGURE 17A & 17B

Transmission electron micrographs of longitudinal section of wheat root-tip meristem (peripheral cells). A. A portion of a control cell showing normal cell wall and plasmalemma 50000X (cf. Figure 16A). B. A portion of a cell exposed to 84.80 μ gU/mL showing relatively high electron density cell wall and pinocytotic vesicles 50000X (cf. Figure 16C).

Figure legend: Cell wall (CW), Plasmalemma (P), Mitochondrion (M), Proplastid (Pp) and Pinocytotic vesicle (PV).



4. DISCUSSION

4.1. Uranium Phytotoxicity:

The results obtained from the study of the germination of wheat, soybean and tomato seeds exposed to a range of uranium concentrations (0.42 to 84.80 $\mu\text{gU/mL}$) demonstrated that uranium did not inhibit germination of these crop species within the range of concentrations used. It also did not affect the dry weight of the seedlings. During seed germination, the metabolism increases rapidly and the substrate for this is derived from the storage reserves (Mayer and Poljakoff-Mayber, 1963; Ching, 1972). The largest amounts of these storage reserves are found in the endosperm of monocots or cotyledons of dicots. These food reserves consist mainly of proteins, lipids and carbohydrates (Ching, 1972). The very fact that dry weight was not affected by uranium treatments suggests that the mobilization of the seed reserves was not impaired.

An early report (Verducci, 1945) showed that radioactivity of uranium compounds was toxic to the germination of seeds. However, the uranium used in the present investigation was natural uranium with very low levels of radiation. Krog (1952) reported a 50% inhibition of germination of red clover and wheat seeds exposed to two orders of magnitude higher concentrations of uranium (compared to the present study) namely 2618 $\mu\text{gU/mL}$ and 5474 $\mu\text{gU/mL}$ respectively. The natural occurrence of such high concentrations of uranium in the environment is highly unlikely (Menzel, 1968; Sheppard and Thibault, 1980; Sheppard et al., 1981). In the present study, low concentrations of uranium were used to simulate the concentrations which may be found in the environment.

The observed decrease in the seedling length of all the three species indicated that uranium was toxic to the early growth of these higher plants, although the rate- and percent germination were unaffected. Specificity of sensitivity to uranium during the early seedling growth was clearly shown. The order of sensitivity from the least to most sensitive species was wheat < soybean < tomato.

The roots were the major sites of uranium toxicity. This was evidenced by a greater reduction in the root length compared to that of shoot length. These results parallel the preceding results of seedling growth, in that tomato was the most sensitive species to uranium treatments perhaps because of the small seed size in comparison to soybean and wheat. Previous studies have shown that uranium was toxic to the root growth. Bombacioni-Mezzetti (1934) reported injury to the roots of plants absorbing uranium from solution containing 50 ugU/mL. Cannon (1952) studying the uptake of ~~uranium~~ and vanadium deposits on the vegetation of the Colorado Plateau reported that excessive concentrations of uranium strongly inhibited root development in higher plants. It has also been reported that Pinus sylvestris seedlings treated with uranium produced thicker, shorter and fewer roots (Sheppard and Thibault, 1980). The toxicity of uranium to the root growth was related to the high concentrations of uranium found in the root system (Cannon, 1952; Sheppard and Thibault, 1980). These, and the present studies demonstrated that the primary target of uranium toxicity is the root system.

The decrease in root length could be due to the observed decrease in cell division of the root meristem and probably also to the inhibitory effects of uranium on cell extension growth as evidenced by

the shortened root hairs. According to an early report by Acqua (1912), uranium absorbed by plant roots was stored as yellow deposits in the cell nuclei of the root meristem. This resulted in the destruction of the chromatin and a cessation of nuclear activity. These effects of uranium observed by Acqua (1912) were related to the radiotoxicity of uranium. This explanation seems unlikely for the presently observed decrease in the mitotic division since no yellow deposits were observed in the nuclei.

The root system plays an important role in the overall physiology of the plant. There is ample evidence that roots contain the major known hormones and perhaps roots serve as the centre for their synthesis (Torrey, 1976). Many of these hormones control the processes of growth such as cell division and cell elongation (Stoddart and Veins, 1980). The environmental influences which affect the root system can adversely act on the water and mineral uptake, the transport of organic substances and also the hormonal balance (Torrey, 1976; Levitt, 1980). Therefore, it is reasonable to assume that uranium may interfere with the phytohormonal balance involved in the cell division and cell elongation processes leading to the observed decrease in the root growth. The decreased root growth induced by uranium may lead to a reduction in the absorption of water and mineral nutrients resulting in the presently observed decrease in shoot growth at uranium concentrations of 42.40 $\mu\text{gU/mL}$ (Murthy *et al.*, 1984).

The seed reserves are hydrolysed during the germination and the products are used by the shoot-root axes for the synthesis of protoplasm, structural components and subsequent growth (Ching, 1972). It is generally agreed that the storage proteins are hydrolysed to amino

acids by proteolytic enzymes. The nitrogen of these amino acids is translocated to the developing shoot-root axes (as amides) and are used to synthesize various enzymes and structural proteins (Ching, 1972; Ashton, 1976). Therefore, the observed (protein) nitrogen content of 6 or 7 d old seedlings, which was not affected by uranium, suggests that the breakdown of proteins in the seed during germination was unaffected during the course of germination.

The protein content in the seeds and seedlings has been related to their vigor. Schweizer and Reis (1969) have shown that those wheat seeds that contained extra proteins, as a result of field application of nitrogen, developed into larger seedlings. In their experiments, the content of protein in the seed correlated with the subsequent growth and yield. The authors suggested that the amount of endogenous protein or protein moiety may be an important factor in subsequent yield of major agronomic crops. In another study, wheat seeds with high protein content germinated faster and developed into larger seedlings compared with those which emerged from average seeds (Lopez and Grabe, 1973). It has also been established that the earliest biochemical event that takes place when the seeds are subjected to unfavourable conditions during germination is the impairment of protein synthesis in barley, wheat (Abdul-Baki, 1969a; 1969b) and soybean (Abdul-Baki and Anderson, 1973). Hence, the observed total (protein) nitrogen in 6 or 7 d seedlings which was unaffected by uranium indicates that the synthesis of proteins and enzymes in the early stages of germination were presumably not affected. However, the total (protein) nitrogen decrease in exposed 10 d old seedlings suggests that subsequent synthesis of proteins and the enzymes paralleled a decrease in growth.

There are no published reports regarding the effects of uranium on the synthesis of proteins either in animal or plant systems. In in vitro studies of precipitation of animal proteins by uranium, Dounce and Lan (1949) reported that pH has an important influence. They used uranyl acetate as the precipitating agent and found that the effects of lowering pH from approximately pH 7.0 to pH 5.0 increased the precipitating action of uranyl acetate on proteins. Dicarboxylic amino acids were shown to be effective in complexing uranium (Strandberg et al., 1981).

Uranium has been shown to affect carbohydrate metabolism in microorganisms. Rothstein et al., (1948) and Rothstein and Meier (1951) have shown that uranium specifically inhibits the metabolism of hexoses forming undissociated but reversible complexes with specific loci on the surface of yeast cells. Rothstein and co-workers (1948; 1951) suggested that the uranium interferes with sugar metabolism by preventing phosphorylation reactions on the cell surface. It has also been reported that uranium inhibits sugar and amino acid uptake by bacteria (Gale, 1954), and sugar utilization in Neurospora (Cochran and Tull, 1958). Singer et al., (1947) provided evidence for the inhibition of several enzymes of carbohydrate metabolism such as hexokinases from yeast, phosphorylase from potato and succino-oxidase from pigeon-breast muscle. They also found that a high concentration of ATP reversed a part of the inhibition of hexokinase, and inorganic phosphate prevented the inhibition of succino-oxidases. These studies (although largely on microorganisms apart from the in vitro study of potato phosphorylase) show that uranium may interfere with the enzymes involved in the metabolism of proteins and carbohydrates affecting seedling growth. The

observed decrease in (protein) nitrogen may also have been indirectly influenced by uranium, in that a decrease in root growth would lead to a decreased absorption of water and mineral nutrients. Mineral ion and water deficiency in plants has been linked with reduced carbohydrate and nitrogen metabolism (Black, 1967; Evans and Wilde, 1971; Nowakowski, 1971).

In the present study the observed reduction in the root growth together with chlorosis, necrosis and decreased chlorophyll content of 4 wk old soybean plants could also be due to the reduction in the absorptive capacity of the roots. Magnesium is a constituent of the chlorophyll molecule (Nason and McElroy, 1963) and magnesium, potassium and iron deficiency results in chlorosis and necrosis of leaves in higher plants (Embleton, 1966; Evans and Sorger, 1966; Wallihan, 1966; Chapman, 1967). Since Mg^{2+} and K^{+} uptake were shown to be decreased by uranium, it can be proposed that reduction in the absorption of mineral ions may lead to the observed phytotoxic response in 4 wk old soybean plants.

These studies demonstrate that uranium is toxic at concentrations $\geq 42.40 \mu gU/mL$ to the early seedling growth of the test crop species, although the rate and percent germination were not affected. The primary target appeared to be the root system. The reduction in root growth would lead to a decrease in absorption of water and mineral nutrients and result in the phytotoxicity to the shoot growth, and chlorophyll content.

4.2. Uranium Uptake:

The results of the study of uranium accumulation in 6 or 7 d wheat, soybean and tomato seedlings demonstrate that uranium is capable of accumulating in the shoot-root axes in large quantities during their germination and early growth. Clear differences have been noted with species as to their ability to accumulate uranium. The greater accumulation of uranium in the shoot-root axes of wheat and soybean is highly significant in view of the observed phytotoxicity to the root and shoot growth.

In the present investigation, an attempt was made to determine the mechanism of biosorption. The determined biosorption curves of all the three species resembled those of adsorption isotherm curves (Giles et al., 1960). The adsorption refers to the existence of a higher concentration of a particular component at the surface of the liquid or solid phase, as opposed to absorption which refers to a more or less uniform penetration. It is not possible in the present case to separate the effects of adsorption from absorption, hence the term biosorption is used. An isotherm represents a relationship between the amount of an environmental chemical (uranium) adsorbed per unit weight of adsorbent (plant material) and the concentration of environmental chemical (uranium) in the solution at equilibrium. The adsorption of environmental chemicals can generally be evaluated by the use of adsorption isotherm models (Giles et al., 1960). The Freundlich model equation is the most widely accepted adsorption isotherm model equation. The Freundlich equations calculated for each set of data have been used as a mathematical function in order to explain the collected

experimental data. Hence, these equations should not be used as general equations. The regression lines drawn for the experimental data points showed that the Freundlich model equation fitted the experimental data points reasonably well (correlation co-efficient 0.997 for wheat, 0.973 for soybean and 0.980 for tomato). This indicates that the biosorption of uranium may be proceeded by physico-chemical adsorption processes. Differences in the correlation coefficients may suggest some differences in the mechanism of physico-chemical adsorption in different species.

The Freundlich equation has been widely used to describe the adsorption in nonbiological systems (Giles et al., 1960). It has also been used to describe the adsorption in biological systems. Chiu (1972) and Tsezos (1980) demonstrated that the Freundlich equation describes the uranium biosorption by Penicillium sp. and Rhizopus arrhizus. They suggested that the surface adsorption was the principal mechanism of uranium biosorption followed by a slower process associated with the transport across the cell membrane. In addition, Tsezos (1980) provided evidence for the adsorption of uranium to the surface of cell wall by electron microscopic studies and x-ray energy dispersion analysis of Rhizopus arrhizus. Rothstein and co-workers (1948; 1951) have shown that uranium acts at the yeast cell surface by complexing with groups which are associated with glucose metabolism. Rothstein and Larrabee (1948) presented conclusive evidence for the adsorption of uranium to the yeast cell surface. It has been suggested that there are reactive groups on the yeast cell surface which are chemically similar to the high molecular weight polyphosphates, and responsible for uranium complexation. In addition accessory groups such as hydroxyl and carboxyl groups can form stable complexes with uranium (Rothstein and Meier, 1951). Rothstein and co-workers (1948; 1951) concluded that uptake of

uranium by yeast cells was biphasic, consisting of a rapid phase whereby uranium formed complexes with one or more functional groups on the cell surface and this followed by a slower phase probably associated with the transfer of uranium across the cell membrane.

It is a well established fact that the primary cell walls of higher plants are mainly made up of cellulose, hemicelluloses and protein (Preston, 1975; Lamport, 1965). In tomato as much as 22% of protein may be present in the cell wall (Lamport, 1969). The cell walls contain a high level of certain amino acids such as hydroxyproline, glutamic acid and aspartic acid (Preston, 1975). It has been shown that the dicarboxylic amino acids such as glutamic acid and aspartic acid can complex with uranium and precipitate as uranium salts (Strandberg et al., 1981). In the leaf tissues of Caprosma australis, more than 50% of uranium was shown to be bound to the cell wall proteins (Whitehead et al., 1971). Cell wall polymers such as cellulose and modified celluloses were reported to be active in binding uranium (Keller et al., 1983).

On the basis of these observations, it can be proposed that the first phase of uranium biosorption may proceed by physico-chemical adsorption by binding to the cellulose and protein content of the cell wall. Evidence can be provided by the transmission electron microscopic studies of wheat root-tip meristems which showed cell walls with relatively high electron density (Figures 14-17).

The observed uranium accumulation in the 4 wk old soybean plants is in agreement with the previous findings (Cannon, 1952; Prister and Prister, 1970; Sheppard and Thibault, 1980) that uranium is preferentially accumulated in the root system. These results are consistent with the fact that uranium forms nondissociable complexes with active groups

on the cell surface (Rothstein and Meier, 1951). In addition, uranium may be bound to the cellulose and protein content of the root cell wall thus being immobilized resulting in the higher accumulation of uranium in the root system than in the shoot system.

The observed uranium content in the shoot system of soybean show that some of the uranium in the root system is in the absorbed state and may be translocated to the shoot system. Prister and Prister (1970) have reported that only 10% of uranium in the root system was in the exchange-absorbed state and 90% was contained in the plant roots and bound to it in a form incapable of exchange. These observations provide evidence for the second phase of uranium biosorption which is involved in the transfer of uranium across the cell membrane.

The accumulation of uranium is not only dependent on the concentration of uranium in the medium, but also on such environmental influences as pH and competing cations. In the present study, the accumulation of uranium was decreased by increasing the concentration of calcium. Cannon (1957) using plants belonging to the pine, rose and legume families studied the biogeochemical aspects of uranium prospecting and found that large amounts of selenium, sulphur and calcium were absorbed along with uranium. Studies on Saccharomyces species (Strandberg et al., 1981) have indicated a reciprocal relationship between Ca^{2+} in the medium and uranium uptake. Rothstein and Meier (1951) showed that bivalent cations such as Ba^{2+} , Ca^{2+} can compete with uranium on the cell surface of yeast. These observations underscore the similarity of mechanism that operate in lower and higher plants by which uranium is modulated by calcium in the medium.

In the present study the uptake of uranium by wheat seedlings was markedly influenced by the pH of the solution. This result is in

close agreement with the previous findings in Chlorella regularis (Nakajima et al., 1979) and yeast cells (Rothstein and Meier, 1951). Uranyl ions are known to form fairly stable complexes with dihydrogen phosphate ion and hydrogen phosphate ions such as $UO_2(H_2PO_3)^-$ or $UO_2(HPO_4)_2^{2-}$ at pH 4.0 and pH 8.0 (Horikoshi et al., 1981; Nakajima et al., 1979). It has also been shown that there are more UO_2OH at pH higher than 7.0, which are further hydrolysed to the poorly soluble $UO_2(OH)_2 \cdot H_2O$ (Pribil and Marvan, 1976). These complexes will not be taken up by cells (Nakajima et al., 1979) leading to the decreased uptake of uranium at pH 4.0, and pH 7.0 to 8.0. At pH 5.0 to 6.0 uranium was present mainly in the form of uranyl ion and was available for the uptake.

The measurement of pH of the germination media indicated that the accumulation of uranium by the roots substantially increased the pH of the medium. These observations suggests the release of hydroxyl ions. However, species specific differences were noted in the uptake of uranium and the changes in pH. Similar differences have been reported for cultures of Scenedesmus quadricauda (Pribil and Marvan, 1976) and Saccharomyces cerevisiae (Strandberg et al., 1981).

The physiological acidity or alkalinity generated in the root bathing medium depends on whether cations or anions are rapidly absorbed by the plants (Moore, 1971). An important process which influences the ionic status of the plant is the uptake of nitrogen. It is absorbed either as nitrate or ammonia. When it is absorbed as nitrate, it has to be balanced either by an equivalent amount of cations or by the release of anions. Such an ion exchange has been proposed for OH^- and HCO_3^- (Kirkby and Mengel, 1967). In most cases potassium accounts for the greater part of the total cation uptake (Collander, 1941; Evans and

Sorger, 1966). The active uptake of K^+ is mainly responsible for balancing the absorbed nitrate (Moore, 1971). In the present study, this could account for the increased pH of the medium containing the untreated control sets of soybean and wheat. The increasing trend in pH with increased uranium concentrations observed in these species indicates an imbalance in the ionic state.

The ionic status of crop plants is not homogenous, and the ionic requirements may be different (Benton-Jones, Jr., 1967). In tomato plants supplied with NH_4^+ ions, the diffusible organic anions were less than from the plants supplied with nitrogen in the form of NO_3^- . During the assimilation of NH_4^+ ions, H^+ are produced (Kirby and Mengel, 1967). Evidence suggests that the release of H^+ is compensated by the uptake of K^+ to maintain the electrical neutrality, thus maintaining the pH (Moore, 1971). Similar mechanisms may operate in the control sets (absence of uranium) containing tomato seeds in which there was no significant change in pH during germination. An increase in pH of the medium was obtained only in the uranium treated sets of tomato again suggesting that this was a result of an imbalance in the ionic status of the uranium treated plants. The presence of large amounts of K^+ approximating 25% of the ash of higher plants (Ulrich and Ohki, 1966), and its role in maintaining the electrical neutrality and osmotic balance (Mengel, 1971; Moore, 1971) strongly suggest that uranium may interfere with the K^+ flux.

The results discussed in this section show that uranium biosorption may be biphasic, the first phase may proceed by adsorption to the cell wall and plasma membrane, and the second phase may involve the transfer of uranium across the cell membrane. The pH of the ambient

medium and the competing cations (Ca^{2+}) markedly affect the biosorption of uranium. In addition, the changes in the pH of the medium suggest that an ionic imbalance may be created during uranium biosorption.

4.3. Ion Flux and Membrane Permeability

The results obtained from the study of electrical conductivity and potassium flux show that there were substantial differences among the three plant species with respect to their K^+ flux.

In soybean, uranium-induced efflux of K^+ was noted, although there was no difference in the total leakage of electrolytes suggesting that a counter ion flux may have been involved (Hiatt and Legget, 1971). The potassium flux showed a clear gradation of uranium effects in tomato. A partial inhibition of net influx at low concentrations of uranium treatments, and a complete suppression of influx and the stimulation of efflux at high uranium treatment levels was evident in tomato. In wheat, the net efflux of K^+ was observed after 8 d germination and early growth at two lower treatments of uranium, while the higher treatments resulted in the efflux of K^+ after 6 d. The insignificant increase between 9 and 10 d in the control sets of wheat may be due to the negative feedback mechanism of K^+ uptake (Bérczi et al., 1982).

These observations demonstrate that the K^+ concentration of the medium of each of the three crop species was either unchanged or increased at the end of the experimental period by two higher uranium concentrations of 42.40 and 84.80 $\mu\text{gU/mL}$. An increased K^+ concentration

indicates that there was a net efflux of K^+ in all the three species after 5 or 6 d germination which may have lead to K^+ deficiency in the seedlings.

Potassium is the most abundant cation in higher plant tissues constituting 1.7 to 2.7 percent of the dry matter (Evans and Sorger, 1966). Chapman (1967) compiled the nutrient values for various elements suggesting low, adequate and high status of normal crop plants. In this compilation the low potassium status of 1.5% was recorded for tomato, and the range of low to high status of 2.3 to 3.4% for wheat (Chapman, 1967), while 2.04 to 2.68% was recorded for soybean (Benton-James, Jr., 1967).

Since there was no net influx of K^+ in the uranium treated sets during the course of germination and early growth, it can be suggested that uranium induces K^+ insufficiency. In addition, 16 d old wheat seedlings had a K^+ content of only 1.25% (321 mM/g dry wt.) which was reduced to an even lower level of 0.7% (180 mM/g dry wt.) in the presence of uranium (Table 17). This would suggest that roots are even more susceptible to uranium induced deficiency than the shoots. Masson et al., (1966) working with barley roots have shown that uranium inhibited K^+ uptake, and suggested that this was probably due to the binding of uranium to the active sites responsible for K^+ flux.

There is substantial evidence in the literature regarding the high affinity of uranium for phosphate groups (Rothstein et al., 1948, Dounce and Lan, 1949; Rothstein and Meier, 1951) and phospholipids constitute an important biological component of cell membranes (Simon, 1974). Thus it can be proposed that uranium may be bound to the plasma membrane. It is evident from the uranium biosorption studies (discussed

in Section 2. Uranium Uptake) that most of the uranium may be bound to the cell wall and the structures close to it such as the plasmalemma. The transmission electron microscopic studies (Figures 14-17) give an additional support for the change in membrane permeability which was visualized by the formation of invaginations and the pinocytotic vesicles arising from the plasmalemma. In addition, Masson et al., (1966) reported that there was a strong retention of uranium by barley root tissues. This conclusion of Masson et al., (1966) was based on the persistent inhibitory effects of uranium on the absorption of K^+ following pretreatment with uranium. Studying the effects of uranium on the metabolism of Baker's yeast, Kotyk et al., (1971) hypothesized that uranium alters the symmetry of the cell membrane transport system. All these observations support the hypothesis that uranium may be bound tightly to the active sites (in the plasmalemma) involved in K^+ flux. This alters the membrane permeability leading to the deficiency of potassium.

Potassium requirement cannot be completely satisfied by any other alkali cations, although in some plant species beneficial effects of Na^+ and Rb^+ have been reported when the K^+ supply was limited (Evans and Sorger, 1966). It has been shown that K^+ insufficiency retarded the utilization of respiratory substrates and blocked protein synthesis (Evans and Wilde, 1971). Evans and Sorger (1966) pointed out that the activities of about fifty enzymes known to participate in various metabolic processes were either completely dependant on, or were stimulated by K^+ . High concentrations of K^+ are also needed to neutralize the anions of the cytoplasm and in this role it contributes to the osmotic potential (Moore, 1971). Chapman (1967) reported that Fe^{2+} and Fe^{3+} mobility may be impaired when K^+ is deficient.

The results discussed in this section show that uranium alters the permeability of the membrane for K^+ . Evidence in the literature suggests that K^+ flux is intimately associated with Na^+ flux via the K^+ influx pump and Na^+ efflux pump (Pitman et al., 1968; Etherton, 1963; Pitman and Staddler, 1967). The results obtained from present set of experiments to determine the effects of uranium on K^+ and Na^+ flux indicated that uranium selectively inhibited K^+ and Mg^{2+} accumulation and stimulated Na^+ accumulation in wheat seedlings. Many higher plants absorb more K^+ than Na^+ , even though the growth medium contains equal concentrations of these ions, or even more Na^+ than K^+ (Collander, 1941; Pitman and Staddler, 1967). Collander (1941) studying the uptake of cations by 20 species of higher plants representing different ecological types and several taxonomic groups has shown that the K^+ to Na^+ ratio was always 3 or higher in all the plants except a few halophytes. In view of the diverse role of K^+ in the metabolism of higher plants, it is not surprising to find such a high concentration of this element (Evans and Sorger, 1966). In the present study, the observed ratio of K^+ to Na^+ (3.5) in the control wheat seedlings (absence of uranium) is in agreement with this generalization. However, in the uranium treated seedlings the ratio of K^+ to Na^+ was reversed (0.41 and 0.35). Pitman and Staddler (1967) studied the K^+ and Na^+ flux in excised barley roots and concluded that the selectivity of K^+ over Na^+ was set up at the plasmalemma. The presently observed reversed ratio (0.41 and 0.35) of K^+ to Na^+ in the uranium treated seedlings suggests that this selectivity at the plasmalemma was altered. The altered permeability of the plasmalemma may

lead to the secondary effects of uranium on enzymes involved in the active transport of K^+ and Na^+ such as various ATPases.

Adenosine triphosphatases (ATPases) have been implicated as energy transfer agents for the transport of inorganic ions in animal and bacterial cells (Skou, 1965; Hokin and Dahl, 1972; Abrams et al., 1972). Plant cells contain several enzymes that are capable of hydrolysing ATP. Several membranes of plant cells also contain ATPase activity and it is quite possible that some of these enzymes are involved in ion transport (Hodges, 1976). It has been shown that uranium inhibits Na^+-K^+ ATPase derived from animal and human tissues (Nechay et al., 1980). The inhibition of this enzyme by uranyl nitrate in animal and human tissues was antagonized by Na^+ suggesting that uranium may inhibit the enzyme at the Na^+ site. Decreased ATP content increased the enzyme inhibition (Nechay et al., 1980).

The observed decrease in total ATP content of wheat roots in the present study is highly significant with regard to the importance of ATP in ion transport. However, it is premature to draw any inference from the present results since many differences in the ATPases of animals and plants have been noted. For instance, the plasmalemma ATPases of oat roots were shown to be quantitatively different from the plasmalemma ATPases of mammalian cells (Hodges, 1976). The plant ATPases are insensitive to Ouabain which is an inhibitor of mammalian ATPases (Fisher and Hodges, 1969; Leonard and Hodger, 1973). Further studies are essential to implicate any interference of uranium with the ATPase enzymes involved in ion transport.

ATP is considered to be the keystone of all cellular activities (Patterson et al., 1970) and serves as a biochemical index of seedling vigor (Abdul-Baki, 1980). The ATP content of the imbibed seeds has been

significantly correlated with seedling size in fatty, starchy and proteinaceous seeds and is indicative of viability in the seed lots (Ching, 1973). The ATP content and adenylate energy charge have also been shown to be related to the growth potential as well as protein and RNA synthesizing ability of wheat seedlings (Ching and Krowstand, 1972). In the present study, uranium induced reduction of ATP content thereby decreasing the seedling vigor.

Hackett (1968) demonstrated that K^+ deficiency reduced primary lateral root formation and totally prevented secondary laterals from emerging in barley roots. Similarly Drew (1975) found that K^+ deficient barley plants had a poorly developed lateral root system. The present observations of both of the intact plants (Figure 1) and of the scanning electron micrographs (SEM) suggest that uranium induced inhibition of K^+ absorption may be responsible for the reduced elongation growth of primary and lateral roots.

The ultrastructural abnormalities observed by TEM of wheat root tip meristem cells following exposure to uranium are consistent with the preferential accumulation of uranium observed in the roots (Tables 14 and 15) and the altered membrane permeability which lead to the decrease in the net influx of K^+ discussed previously.

The relatively high electron density of the cell wall supports the view that most of the uranium may be bound to the cellulose and proteins of the cell wall. The functional groups capable of serving as binding sites for uranium may include virtually any anionic centre and a variety of ligands capable of co-ordinating with uranium (Keller et al., 1983).

The uranium induced plasmalemma dilations, invaginations and the pinocytotic vesicles are in line with the hypothesis that uranium may be bound to phosphate components of the plasmalemma. This binding of uranium to the plasmalemma alters the membrane permeability for K^+ absorption. As noted before, the differential effect of uranium on K^+ and Na^+ accumulation suggest that uranium may be bound to the active sites involved in K^+ absorption and alter the selectivity of the plasmalemma for K^+ .

The observed absence of any ultrastructural changes in the subcellular organelles such as nuclei, mitochondria and proplastids in the uranium treated cells are interesting in view of the similarities in the chemical composition and the structure of the plasmalemma, and the membranes of these subcellular organelles (Frey-Wyssling and Muhlethaler, 1965). These results indicate that few uranyl ions may be transported to the cytoplasm where concentration of uranium may not reach the toxic level needed to induce such changes as observed in the plasmalemma. The apparent normality of mitochondria suggests that the observed reduction in ATP content is not a result of impaired mitochondrial function.

Wheeler and Hanchy (1971) and Robards and Robb (1972) have reported the pinocytotic uptake of uranium in isolated root cells of oat and barley using a high concentration of 1mM uranyl acetate. Similarities were noted between these and the present study. However, Wheeler and Hanchy (1971) and Robards and Robb (1972) have shown the heavy deposition of uranium crystals in the cell wall and packets of uranium crystals which were membrane bound in the cytoplasm. Such

structures were not found in the present investigation (Figures 14-17). This may have been due to the use of intact roots and the low environmentally relevant concentration (84.80 $\mu\text{gU/mL}$) of uranium in the present investigation.

4.4. Relevance to Field Conditions:

A review of the literature concerning contamination by uranium of the environment revealed that the concentration of uranium has been increasing as a result of the activity of the nuclear industry and the use of phosphate fertilizers. For example, Sheppard and Thibault (1980) recorded a concentration of 26 $\mu\text{gU/g}$ soil in one of the nuclear waste dump sites. The soils and tailing materials around three mining and milling areas were shown to contain uranium concentrations ranging from 3 to 450 $\mu\text{gU/g}$ soil ash (Sheppard et al., 1981). In addition, the radioactivity of the fertilizer material made from phosphate rocks obtained from different parts of the United States was reported to contain 35 to 108 Ci in 1974. The total radioactivity calculated for that year (in the United States) from phosphate fertilizers was 1160 Ci (Guimond, 1978). The phosphate rocks used in phosphate fertilizers have been shown to contain an average of 90 $\mu\text{gU/g}$ with a maximum of 400 $\mu\text{gU/g}$ (Mazor, 1963; Menzel, 1968). The uranium concentrations at two depths of soils collected from the area of agricultural fields around Savanna River Plant, South California, were reported to contain 579 ± 164 and 602 ± 119 pCi/g dry weight (Smith et al., 1981).

The data obtained from the present study indicated that uranium concentrations of 0.42 and 0.84 $\mu\text{gU/mL}$ were not phytotoxic to the crop species tested. Uranium concentrations of 4.24 and 8.48 $\mu\text{gU/mL}$ were

moderately phytotoxic, and 42.40 and 84.80 $\mu\text{gU/mL}$ were highly phytotoxic to the early growth of the plants. Hence, it can be concluded that environmentally relevant concentrations of uranium used in the present study are phytotoxic. In view of the expected expansion of the nuclear energy programme and agricultural dependence on fertilizers, there will likely be an added increase in the mobilization and dispersal of uranium. In addition, the relatively long half-life of uranium (4.50×10^9 yrs for U^{238}) also contribute to the dangers of uranium presence in the environment.

It is recognized that direct correlation cannot be made from laboratory studies to the field situation, as multitude of abiotic and biotic factors in the environment may influence the behavior of uranium. Sheppard (1980) summarized the literature concerning the environmental behavior of uranium. She noted that the concentration of uranium in the test plants depends on the mode of occurrence of uranium in the soil and water, physiology and species of plants, time of the year and the soil availability of the element.

Species related differences in the ability to accumulate uranium and sensitivity to its effect were demonstrated in the present study. The mode of biosorption was shown to proceed by physico-chemical adsorption process. However, further studies are warranted to determine the exact mechanism of uranium transfer across the membrane and mobility within the plant which accounts for about 10% of uranium in the shoot system.

The present study also shows that uranium interferes with the uptake of certain cations such as K^+ and Mg^{2+} by altering the membrane permeability. Further studies can throw some light on the probable

mechanism of uranium induced inhibition of K^+ uptake and the probable involvement of specific enzymes responsible for cation absorption.

The decrease in ATP content presently noted is interesting in view of the high affinity of uranium for phosphate groups (Rothstein et al., 1948; Rothstein and Meier, 1951). Perhaps uranium and phosphate ions present in the germination solution may form uranyl phosphate complexes which are unavailable for plant uptake. This may result in the deficiency of phosphate which in turn leads to the decreased ATP content. All these observations point in the direction of interaction of mineral ions with uranium uptake. Future studies are essential to establish the role of these ions in uranium uptake and its toxicity.

Although, one cannot directly extrapolate these laboratory studies to field conditions, the present study does provide useful information relating to some environmental factors that may affect uranium bioavailability, and the possible mode of biosorption and of phytotoxicity.

The uranium mining and milling industry is rapidly expanding in Canada, especially in Saskatchewan, and the approximate projections indicate that 400 million pounds of uranium oxide may be produced between now and the year 2000. The processing of the ore in uranium mills would generate approximately 10 million tons of finely ground waste solids called "tailings" (Environment Canada Report, 1981). The federal and provincial environmental protection requirements, which regulate the uranium mining industry in Saskatchewan today, did not exist in the late 1950s or early 1960s. Therefore, the uranium mills which operated in Saskatchewan in those years discharged the tailings into small depressions or lakes without much regard for environmental protection. Hence waste management considerations must be given to

containing and monitoring the leaching of uranium throughout watersheds. Bioaccumulation of uranium should be closely monitored in root crops (carrot, potato) grown in soils directly or indirectly exposed to uranium.

Revegetation studies of mine abandonment is presently being undertaken in Saskatchewan. The present study underlines the fact that joint industry-university studies should be undertaken to establish the type of vegetation most suitable for use in infilling sites in order to sequester the largest amount of uranium in the vegetation and thus reduce the concentration leaching to ground waters.

The effect of Ca^{2+} on reducing uranium uptake observed in the present study may offer a way by which biotic sorption by crop plants may be reduced. Liming areas around mine slags and adding lime to phosphate slag used for wad infilling and phosphate fertilizers may make these areas more environmentally acceptable.

Monitoring levels of uranium in natural phosphate rocks used for fertilizers should lead to the identification of areas where levels are high. These should be earmarked for restricted use. Apart from potential health hazards, this research shows that cost-benefit studies may indicate that adding phosphate rock fertilizers to sensitive crops may actually diminish total yield.

SUMMARY AND CONCLUSIONS

The results presented in this thesis demonstrated that uranium in the range of concentrations of 0.42 to 84.80 $\mu\text{gU/mL}$ did not affect germination, but was toxic to the subsequent growth of wheat, soybean and tomato seedlings grown under hydroponic conditions.

The test crop plants sorbed significant amounts of uranium at all levels of treatments. Exposed to 84.80 $\mu\text{gU/mL}$, soybean, wheat and tomato accumulated 243, 569 and 909 $\mu\text{gU/g}$ dry wt respectively. The shoot-root axis was the preferential site of uranium accumulation. The amount of uranium accumulated in this organ compared to that in the food storage tissues (cotyledons or endosperm) was 3:1. However, the germination process as defined by the speed and the total percent germination and total (protein) nitrogen content was unaffected by any level of treatment. Uranium treatments in the range tested apparently did not affect the mobilization of food reserves required for seedling emergence, but the subsequent growth reduction was evident.

The root was the major site of uranium accumulation and toxicity. The ratio of sorbed uranium in the root:shoot was as 10:1. Sorption of uranium into the plants was by physicochemical adsorption process as demonstrated by Freundlich model adsorption isotherms, sorbing to the cell wall and to the plasmalemma. Sorption was modified by pH. Within the physiological range of accumulation, it was optimal at pH 5.0 to pH 6.0, and minimal at pH 4.0 and pH 8.0. Additional Ca^{2+} reduced the uptake of uranium.

Uranium inhibited the influx of K^+ and stimulated its efflux. In addition, the selectivity of K^+ over Na^+ at the plasmalemma was altered from 3:1 ratio in the controls to 1:3 in plants exposed to 84.80 $\mu gU/mL$. This was accompanied by a decreased accumulation of magnesium.

Seedling vigor was reduced by uranium treatments $\leq 4.24 \mu gU/mL$ in tomato, 8.48 $\mu gU/mL$ in soybean and 42.40 $\mu gU/mL$ in wheat. This was reflected in a decreased seedling length and the decreased (protein) nitrogen content of all the three species, and decreased root ATP content of wheat. The mitotic index of the root tip meristem was also adversely affected.

Transmission electron microscopic studies confirmed the observations of uranium adsorption to cell wall and plasmalemma, and an altered permeability of the membrane which was visualized by the heavy labelling of the root cell wall and the formation of plasmalemma dilations and pinocytotic vesicles derived from the plasmalemma. In addition, scanning electron microscopic studies showed the growth inhibition of root hairs and lateral roots.



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APPENDIX I

Effects of uranium on the % germination and germination values of wheat

Treatments μgU/mL	% germination	Germination Value (GV)	
		MDG	X PV
Control	88 ± 6 a*	297 ± 29	a
0.42	87 ± 11 a	308 ± 43	a
0.84	87 ± 9 a	306 ± 44	a
4.24	85 ± 9 a	283 ± 40	a
8.48	85 ± 14 a	279 ± 47	a
42.40	84 ± 9 a	269 ± 61	a
84.80	83 ± 12 a	264 ± 48	a

MDG = Mean Daily Germination

PV = Peak Value

GV = Germination Value

(See Czabator, 1962)

* Means (n = 500) with standard deviations followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

APPENDIX II

Effects of uranium on the % germination and germination values of soybean

Treatments μgU/mL	% germination	Germination Value (GV) MDG X PV
Control	90 ± 5 a*	375 ± 40 a
0.42	86 ± 7 a	375 ± 31 a
0.84	87 ± 8 a	367 ± 30 a
4.24	90 ± 4 a	286 ± 60 a
8.48	86 ± 3 a	280 ± 75 a
42.40	79 ± 8 a	293 ± 71 a
84.80	80 ± 6 a*	281 ± 46 a

MDG = Mean Daily Germination

PV = Peak Value

GV = Germination Value

(See Czabator, 1962)

* Means (n = 500) with standard deviations followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

APPENDIX III

Effects of uranium on the % germination and germination values of tomato

Treatments μgU/mL	% germination	Germination Value (GV) —MDG X PV
Control	97 ± 2 a*	325 ± 12 a
0.42	96 ± 3 a	332 ± 17 a
0.84	95 ± 3 a	310 ± 17 a
4.24	95 ± 2 a	304 ± 18 a
8.48	93 ± 2 a	296 ± 21 a
42.40	93 ± 3 a	299 ± 15 a
84.80	92 ± 2 a	286 ± 18 a

MDG = Mean Daily Germination
PV = Peak Value
GV = Germination Value

(See Czabator, 1962)

* Means (n = 600) with standard deviations followed by the same letter are not significantly different at P<0.05. level. (Tukey, 1949).

APPENDIX IV

Effect of uranium on shoot length (cm) of wheat (6 d)
after the completion of germination

Treatments $\mu\text{gU/mL}$	Group mean $\pm$ S.E.	% of control
Control	8.33 $\pm$ 0.45 a*	
0.42	8.92 $\pm$ 0.44 a	106
0.84	8.51 $\pm$ 0.44 a	101
4.24	8.58 $\pm$ 0.45 a	102
8.48	8.38 $\pm$ 0.42 a	100
42.40	8.03 $\pm$ 0.40 a	96
84.80	7.24 $\pm$ 0.38 a	86

* Means ($n = 300$) with standard error followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).

APPENDIX V

Calculation of the Numerical Values in the Freundlich Equation:

Wheat: From the 6 sets of experimental data ($\frac{x}{m}$ vs C') $\frac{x}{m}$ represents μg uranium biosorption per gram dry wt of the plant material and C' the external environmental concentration of uranium in the solution (in $\mu\text{gU/mL}$)

C' : 0.324, 0.685, 3.69, 7.48, 40.03, 79.69

$\frac{x}{m}$ = 10.58, 17.96, 60.21, 109.21, 260.21, 565.21

The calculation is proceeded as follows:

$(\log C = x)$	$(\log \frac{x}{m} = y)$	xy	x^2	y^2
-0.489	1.024	-0.500736	0.239121	1.048576
-0.164	1.254	-0.205656	0.026896	1.572516
0.567	1.779	1.008693	0.321489	3.164841
0.874	2.038	1.781212	0.763876	4.153444
1.605	2.415	3.876075	2.576025	5.832225
1.901	2.752	5.231552	3.613801	7.573504
$\Sigma x=4.294$	$\Sigma y=11.262$	$\Sigma xy=11.19114$	$\Sigma x^2=7.541208$	$\Sigma y^2=23.345106$

wheat cont.

$$S_{xy} = \sum xy - \frac{(\sum x)(\sum y)}{n} = 11.19114 - \frac{(4.294)(11.262)}{6} = \underline{3.131302}$$

$$S^2_x = \sum x^2 - \frac{(\sum x)^2}{n} = 7.541208 - \frac{18.438436}{6} = \underline{4.468135}$$

$$S^2_y = \sum y^2 - \frac{(\sum y)^2}{n} = 23.345106 - \frac{126.832644}{6} = \underline{2.206332}$$

$$b = \frac{S_{xy}}{S^2_x} = \frac{3.131302}{4.468135} = \underline{0.700807}$$

$$a = \bar{y} - b(\bar{x}) = 1.877 - 0.700807(0.715667)$$

$$= \underline{1.375}$$

$$y = a + bx = 1.375 + 0.701x$$

$$\text{ie, } \log \frac{x}{m} = 1.375 + 0.701x$$

$$\text{or } \frac{x}{m} = 23.739C^{0.701}$$

This is the Freundlich equation which expresses the relationship between $\frac{x}{m}$ and C' as shown in Fig. 3 for wheat.

The correlation coefficient is

$$r = \frac{S_{xy}}{\sqrt{S^2_x} \sqrt{S^2_y}} = \frac{3.131302}{3.139776} = \underline{0.997}$$

Soybean:

$$C' = 0.163, 0.395, 1.391, 2.909, 24.866, 62.746$$

$$\frac{x}{m} = 2.94, 4.58, 29.63, 59.50, 190.43, 240.83$$

$(\log C = x)$	$(\log \frac{x}{m} = y)$	xy	x^2	y^2
-0.788	0.468	-0.368784	0.620944	0.219024
-0.403	0.661	-0.266383	0.162409	0.436921
0.143	1.457	0.208351	0.020449	2.122849
0.464	1.775	0.8236	0.215296	3.150625
1.396	2.279	3.181484	1.948816	5.193841
1.790	2.382	4.282836	3.232804	5.673924
$\Sigma x = 2.61$	$\Sigma y = 9.022$	$\Sigma xy = 7.861104$	$\Sigma x^2 = 6.200718$	$\Sigma y^2 = 16.797184$

$$S_{xy} = \Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n} = 7.861104 - \frac{(2.61)(9.022)}{6} = \underline{3.926534}$$

$$S^2_x = \Sigma x^2 - \frac{(\Sigma x)^2}{n} = 6.200718 - \frac{6.8121}{6} = 5.065368$$

$$S^2_y = \Sigma y^2 - \frac{(\Sigma y)^2}{n} = 16.797184 - \frac{81.396484}{6} = \underline{3.231103}$$

$$b = \frac{S_{xy}}{S^2_x} = \frac{3.926534}{5.065368} = \underline{0.777147}$$

Soybean cont.

$$a = \bar{y} - b(\bar{x}) = 1.503667 - 0.777147 = 1.165608$$

$$y = a + bx = 1.166 + 0.777x$$

$$\text{ie., } \log \frac{x}{m} = 1.166 + 0.777x$$

$$\text{or } \frac{x}{m} = 14.642C^{0.777}$$

This is the Freundlich equation which expresses the relationship between $\frac{x}{m}$ and C' as shown in Fig. 3 for soybean.

The correlation coefficient is

$$r = \frac{S_{xy}}{\sqrt{S^2_x} \sqrt{S^2_y}} = \frac{3.936534}{4.045581} = \underline{0.973}$$

Tomato:

$$C' = 0.373, 0.771, 3.988, 7.930, 41.630, 83.262$$

$$\frac{x}{m} = 31.33, 51.25, 139.95, 324.95, 481.45, 904.15$$

$(\log C = x)$	$(\log \frac{x}{m} = y)$	xy	x^2	y^2
-0.428	1.496	-0.640288	0.183184	2.238016
-0.113	1.709	-0.193117	0.012769	2.920681
0.601	2.146	1.289746	0.361201	4.605316
0.899	2.512	2.258288	0.808201	6.310144
1.619	2.683	4.343777	2.621161	7.198489
1.920	2.956	5.675526	3.6864	8.737936
$\Sigma x = 4.498$	$\Sigma y = 13.502$	$\Sigma xy = 12.733926$	$\Sigma x^2 = 7.672916$	$\Sigma y^2 = 32.010582$

$$S_{xy} = \Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n} = 12.733926 - \frac{(4.498)(13.502)}{6} = \underline{2.611927}$$

$$S^2_x = \Sigma x^2 - \frac{(\Sigma x)^2}{n} = 7.672916 - \frac{20.232004}{6} = \underline{4.300915}$$

$$S^2_y = \Sigma y^2 - \frac{(\Sigma y)^2}{n} = 32.010582 - \frac{182.304004}{6} = \underline{1.626581}$$

$$b = \frac{S_{xy}}{S^2_x} = \frac{2.611927}{4.300915} = 0.607956$$

Tomato cont.

$$a = \bar{y} - b(\bar{x}) = 2.250333 - 0.607956(0.749667) \\ = \underline{1.795094}$$

$$y = a + bx = 1.795 + 0.607x$$

$$\text{ie, } \log \frac{x}{m} = 1.795 + 0.607x$$

$$\text{or } \frac{x}{m} = 62.387C^{0.607}$$

This is the Freundlich equation which expresses the relationship between $\frac{x}{m}$ and C' as shown in Fig. 3 for tomato.

The correlation coefficient is

$$r = \frac{S_{xy}}{\sqrt{S^2_x} \sqrt{S^2_y}} = \frac{2.611927}{2.664496} = \underline{0.980}$$

APPENDIX VI

Accumulation of uranium ($\mu\text{gU/g}$ dry Wt) in 6 d old soybean seedlings
(original uranium accumulation data)

Treatments $\mu\text{gU/mL}$	Means $\pm$ S.D.
Control	2.17 $\pm$ 1.1
0.42	5.11 $\pm$ 1.7
0.84	6.75 $\pm$ 1.4
4.24	31.80 $\pm$ 7.0
8.48	61.67 $\pm$ 12.0
42.40	192.60 $\pm$ 41.0
84.80	243.00 $\pm$ 75.0

Means of 6 replicates are presented with standard deviations

APPENDIX VII

Accumulation of uranium ($\mu\text{gU/g}$ dry Wt) in 6 d old wheat seedlings
(original uranium accumulation data)

Treatments $\mu\text{gU/mL}$	Means $\pm$ S.D.
Control	3.79 $\pm$ 1.9
0.42	14.37 $\pm$ 4.5
0.84	21.75 $\pm$ 4.7
4.24	64.00 $\pm$ 21.2
8.48	113.05 $\pm$ 39.0
42.40	264.00 $\pm$ 38.0
84.80	569.00 $\pm$ 126.0

Means of 6 replicates are presented with standard deviations

APPENDIX VIII

Accumulation of uranium ($\mu\text{gU/g dry Wt}$) in 7 d old tomato seedlings
(original uranium accumulation data)

Treatments $\mu\text{gU/mL}$	Means $\pm$ S.D.
Control	4.75 $\pm$ 2.9
0.42	36.08 $\pm$ 14.6
0.84	56.00 $\pm$ 17.0
4.24	144.70 $\pm$ 62.0
8.48	329.70 $\pm$ 86.0
42.40	486.20 $\pm$ 125.0
84.80	909.00 $\pm$ 193.0

Means of 6 replicates are presented with standard deviations

APPENDIX IX

Effect of uranium on total ATP Content ($\mu\text{g/g}$ dry Wt)
of wheat roots (Original ATP data).

Treatments $\mu\text{gU/mL}$	day 2	day 4	day 6	day 8	day 10
Control	93 $\pm$ 9 a*	103 $\pm$ 16 a	105 $\pm$ 15 a	88 $\pm$ 3 a	82 $\pm$ 4 a
4.24	111 $\pm$ 2 a	101 $\pm$ 5 a	67 $\pm$ 5 b	49 $\pm$ 2 b	45 $\pm$ 3 b
8.48	101 $\pm$ 10 a	82 $\pm$ 9 a	45 $\pm$ 3 bc	38 $\pm$ 3 c	34 $\pm$ 7 c
42.40	119 $\pm$ 7 a	103 $\pm$ 7 a	42 $\pm$ 4 bc	34 $\pm$ 4 c	30 $\pm$ 4 c
84.80	113 $\pm$ 10 a	72 $\pm$ 11 b	37 $\pm$ 10 bc	28 $\pm$ 2 cd	21 $\pm$ 2 d

* Means ($n = 4$) with standard deviations followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).

APPENDIX X

Effect of uranium on fresh and dry weights
(mg/seedling) of wheat seedlings 6 d Post-treatment

Treatments μgU/mL	Fresh weight	Dry weight
Control	182 ± 52 a*	16 ± 5 a
4.24	177 ± 46 a	14 ± 5 a
8.48	167 ± 62 a	14 ± 4 a
42.40	165 ± 48 a	18 ± 3 a
84.80	165 ± 43 a	18 ± 4 a

* Means (n = 100) with standard deviations followed by the same letter are not significantly different at P<0.05 level (Tukey, 1949).

APPENDIX XI

Effect of uranium on fresh and dry weights
(mg/seedling) of soybean seedlings 6 d Post-treatment

Treatments $\mu\text{gU/mL}$	Fresh weight	Dry weight
Control	978 $\pm$ 170 a*	182 $\pm$ 29 a
4.24	1059 $\pm$ 118 a	191 $\pm$ 23 a
8.48	925 $\pm$ 216 a	188 $\pm$ 17 a
42.40	869 $\pm$ 182 a	185 $\pm$ 24 a
84.80	758 $\pm$ 142 a	187 $\pm$ 25 a

* Means (n = 100) with standard deviations followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).

APPENDIX XII

Effect of uranium on fresh and dry weights
(mg/seedling) of tomato seedlings 7 d Post-treatment

Treatments $\mu\text{gU/mL}$	Fresh weight	Dry weight
Control	$51 \pm 4 \text{ a}^*$	$1.8 \pm 0.06 \text{ a}$
4.24	$56 \pm 4 \text{ a}$	$1.8 \pm 0.03 \text{ a}$
8.48	$49 \pm 5 \text{ a}$	$1.6 \pm 0.20 \text{ a}$
42.40	$49 \pm 6 \text{ a}$	$1.6 \pm 0.30 \text{ a}$
84.80	$48 \pm 3 \text{ a}$	$1.7 \pm 0.20 \text{ a}$

* Means ($n = 100$) with standard deviations followed by the same letter are not significantly different at $P < 0.05$ level (Tukey, 1949).