Concentration of Chromium, Nickel, and Vanadium in Plant Materials

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By adding V and Ni to the culture nutrient solution the V and Ni concentrations in the plants investigated were increased substantially. Vanadium had no particular affinity for natural biogenic fats and oils, nor was it found in high concentration in commercial vegetable oils. The concentrations of Cr, Ni, and V in wheat seed indicate that the concentrations of these elements are influenced by geochemical factors; thus, it should be possible to map areas where plant seeds would contain different amounts of these elements.

In recent years, scientists have found that several trace elements heretofore not believed essential to life are required for the optimum growth and longevity of animals and humans (Frieden, 1972; Scott, 1972). Included among these newer trace elements are chromium (Cr), vanadium (V), and nickel (Ni), but little is known about their distribution in the biosphere. In general, foods and feeds are the major pathway of trace elements into the food chain. Thus, the concentration of Cr, V, and Ni in edible plant products may greatly concern nutritionists interested in trace element requirements of animals and humans.

Sometimes, the concentration of trace elements in plant materials (e.g., Co, Se, and Mo in forage crops) can be related to geochemical properties of the soil on which they grow (Kubota and Allaway, 1972). However, various environmental and genetic parameters interact with soil properties, and these interactions may drastically influence the concentration of a particular element in a plant or plant food product.

In the series of experiments reported here we studied the concentrations of Cr, Ni, and V in various plant species and the factors that affect their concentration because of their probable essentiality to animals and man. The possibilities of geographical distribution patterns and varietal differences in the concentrations of these elements in wheat seeds from major growing areas in the United States are discussed. The concentration of V in various types of vegetable oils was also determined.

MATERIALS AND METHODS

Solution Culture Studies. *Vanadium.* Seeds of barley (*Hordeum vulgare,* cv. Erie), oats (*Avena sativa,* cv. Orbit), pea (*Pisum sativum,* cv. Miragreen), wheat (*Triticum vulgare,* cv. Avon), romaine lettuce (*Lactuca sativa,* subsp. *longifolia,* experimental line C898C, Department of Vegetable Crops, Cornell University), tomato (*Lycopersicon esculentum,* cv. Fireball), flax (*Linum usitatissimum,* cv. Redwood-65), rape (*Brassica napus,* cv. Zephyr), and two varieties of safflower (*Carthamus tinctorius,* cv. Gila and UC-1) were germinated for 3 days in the dark and then transferred to a dilute (1:5 with water) nutrient solution (Johnson et al., 1957) for 7 days. Then plants were grown in a full strength nutrient solution (0.25 μg/ml of V as NH_4VO_3) with iron supplied as the EDTA (ethylenediaminetetraacetate) salt, in the greenhouse in four 32-l. plastic tanks. There were 10 plants per tank (one plant of each variety). The solutions were changed weekly. All plants except lettuce and tomatoes were grown to maturity, and the mature seeds were harvested. The tops of the lettuce and tomato plants were harvested 41 days after germination.

All plant material was dried in a forced draft oven at 65° for 3 days and the unground plant material was analyzed for V by a catalytic method (Welch and Allaway, 1972). Analysis of the NBS standard reference material 1571 (orchard leaves) by this method gave a value of 0.361 ± 0.009 ppm of V. The oil in the ground flax, rape, and safflower seeds was extracted with a mixture of chloroform–methanol–H_2O (1:2:8, v/v/v). The chloroform fraction was separated and then evaporated to dryness on a steam bath. The oil content of the seeds was determined by weighing the chloroform residue.

Nickel. Tobacco (*Nicotiana tabacum,* cv. Coker 319) seeds were germinated for 7 days in the dark on cheesecloth in 0.2 m*M* $CaSO_4$. Then they were transferred to the light and to a one-fifth strength nutrient solution described previously, except Fe was supplied as the EDDHA [ethylenediaminedi(*o*-hydroxyphenyl acetate)] salt at a level of 0.5 ppm of Fe in solution.

Twenty-four days after germination, 40 tobacco seedlings were transferred to a growth chamber with a growth regime of 30°/19° day–night temperatures, 1-hr days, and 1200 ft-c light intensity. After 12 days, 16 of the 40 tobacco seedlings were transferred to four 3.2-l. plastic pots containing half-strength nutrient solutions (Johnson et al., 1957). After 67 days, the plants were transferred to four 32-l. plastic tanks (four plants per tank) containing full strength nutrient solutions. The nutrient solutions were then treated with $Ni(NO_3)_2$ to give 0.05, 0.1, 0.5, or 1 ppm of Ni. The nutrient solutions were changed weekly. After 15 days, the tops were harvested, dried in a forced draft oven at 65° for 48 hr, and ground in a Wiley mill to pass a 20-mesh stainless steel screen. Subsamples (1 g) of the ground tobacco leaf material from each Ni treatment were sulfate ashed (Johnson and Ulrich, 1959) in a muffle furnace at 450° for 24 hr. After ashing, the residues were dissolved in 1 ml of concentrated HNO_3, taken to dryness on a steam bath, and reashed at 500° for 30 min. The residue was dissolved in 1 ml of concentrated HNO_3 and, after making to volume, the diluted solutions were assayed for Ni by atomic absorption spectrophotometry. Recoveries of standard Ni additions to samples before ashing averaged 90%.

Regional Study. Wheat seed was collected from breeding nurseries and test seedings at harvest time, the unground seeds were washed in distilled water, and chromium was determined by the method of Cary and Allaway (1971). Analysis of the NBS standard reference material 1571 (orchard leaves) by this method gave a value of 2.47 ± 0.14 ppm of Cr as compared to the certified value of 2.6 ± 0.2 ppm of Cr. Vanadium was determined by the method mentioned earlier, and Ni was determined by the method of Nomoto and Sunderman (1970). Analysis of the NBS standard reference material 1571 (orchard leaves) by this method gave a value of 1.28 ± 0.17 ppm of Ni as compared to the certified value of 1.3 ± 0.2 ppm of Ni.

Vanadium in Vegetable Oils. Ten types of commercial vegetable oils were obtained from local markets. Oil samples weighing 0.2 g were combusted in an O_2 atmosphere

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Table I. Vanadium Concentration in Seeds and Tops of Various Plant Species Grown in Nutrient Solution with and without Vanadium Additions

Plant species	No V added	V added[a]
	V in Seeds, ppb[b]	
Barley	28	175
Oat	55	151
Pea	54	75
Wheat	46	137
	V in Tops, ppb[b]	
Lettuce	165	780
Tomato	149	844

[a] Vanadium added as NH_4VO_3 at a rate to give 0.25 μg of V/ml in solution weekly. [b] Mean values of two replicate analyses.

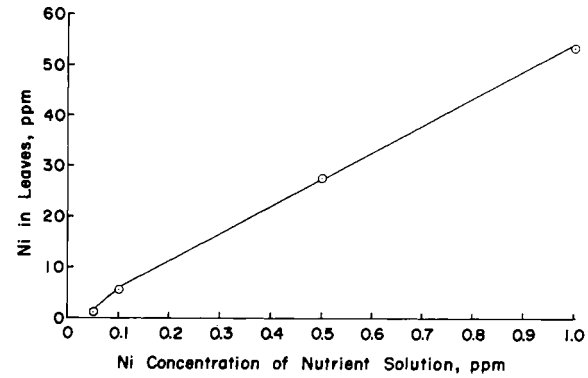

Figure 1. Concentration of Ni in tobacco leaves as affected by Ni concentration in culture solution.

Table II. Vanadium and Oil Concentration in Seeds from Plants Grown in Nutrient Solutions with and without Vanadium Added

			V concn, ppb	
Plant seed	Variety	Oil, %	No V added	V added[a]
Flax	Redwood-65	28	18	102
Rape	Zephyr	20	18	132
Safflower	Gila	29	21	184
Safflower	UC-1	23	19	173

[a] Vanadium added as NH_4VO_3 at a rate to give 0.25 μg of V/ml.

Table III. Vanadium Concentration in Commercial Vegetable Oils

Oil type	V, ppb[a]
Corn germ, cold-pressed	42
Linseed, cold-pressed	14
Olive, cold-pressed	125
Peanut, cold-pressed	103
Safflower, cold-pressed	60
Soybean, cold-pressed	95
Corn, extracted	119
Peanut, extracted	108
Safflower, extracted	106
Soybean, extracted	139

[a] Recovery of V added to oil samples before oxygen flask combustion was 90%.

in a Shöniger flask. Cellulose powder (0.5 g) was added to each sample to facilitate combustion. The combusted residue was dissolved in 50 ml of 0.1 N HCl and its V concentration determined as described above. Percent recovery of V standards added to oil samples, before oxygen flask combustion, was 90%.

RESULTS

The results in Tables I and II are from the experiment with various plant species grown in nutrient solutions with (0.25 μg of V/ml) and without V additions. Vanadium concentration in plant seeds and tops increased as V was added to the nutrient solutions. The highest V concentrations, 780 and 844 ppb of V, were found in tops of the V-treated lettuce and tomato plants, respectively. Generally, the seeds accumulated a relatively low concentration of V, even when grown in the V-supplemented solutions.

The oil content of the oil seeds (i.e., flax, rape, and safflower) ranged from 20 to 29% depending on the seed variety (Table II). The V concentration in the commercial vegetable oils ranged from 14 to 139 ppb of V (Table III).

Figure 1 shows the Ni concentration in tobacco plants grown in nutrient solutions to which from 0.05 to 1.0 ppm of Ni had been added. The concentration of Ni in the tobacco was a linear function of the solution Ni concentration from 0.1 to 1.0 ppm of Ni.

The concentrations of all three elements (Cr, Ni, and V) in wheat seeds from various locations are shown in Table IV. The data suggest small varietal differences in the concentration of these elements in wheat seed. The Cr concentration ranged from 3 to 43 ppb for all samples. Wheat samples from Kansas contained the lowest mean Cr concentration (5 ppb) and those from Montana and South Dakota contained the highest mean concentration of Cr (30 ppb). Wheat seed collected in Kansas had the highest mean Ni concentration (230 ppb) and seed from Montana had the lowest mean Ni concentration (99 ppb). Two-thirds of the seeds analyzed for V contained less than 6.5 ppb of V. Seed from Quinn and Presho, S.D., and Bushland and Denton, Tex., contained relatively more V than seed from the other locations.

DISCUSSION

When 0.25 μg/ml of V concentration was added to the nutrient solution, the V concentration in the tops and seeds of the various plant species increased substantially (Tables I and II). Most freely drained soils contain less than 0.1 ppm of acetic acid extractable V (Mitchell, 1971). The V concentrations in plants grown in the solutions containing added V (Tables I and II) represent plant tissues cultured in nutrient solution containing relatively high nontoxic levels of V (Pratt, 1966). Therefore, they are much higher (Tables I and II) than V concentrations of wheat seeds grown on various soil types (Table IV).

Since V is reportedly concentrated in some shales (Rankama and Sahama, 1950), this may explain the relatively high V concentrations in wheat seed from soil developed from the Pierre shale in South Dakota.

Schroeder et al. (1963) found that V has an affinity for all natural biogenic fats and oils and reported V concentrations as high as 43.53 ppm of V in crude cold-pressed soybean vegetable oil. However, other investigations (Söremark and Ullberg, 1961; Söremark, 1967) have failed to confirm this. The data presented in Tables II and III support the results obtained by Söremark and his coworkers. High V concentrations were not found in any of the various oil seeds (Table II) and commercial vegetable oils (Table III) analyzed, even though some of the flax, rape, and safflower seeds (Table II) were harvested from plants grown in solutions of relatively high V concentrations.

Of the three elements studied, only Ni accumulates to any appreciable extent in plant seeds. In solution culture,

Table IV. Mean Concentration of Cr, Ni, and V in Wheat Seed

Soil type	Surface pH	Variety[a]	ppb Cr[b]	ppb Ni[c]	ppb V[c]
Bozeman, Mont.					
Sicl	6.8–7.0	Centana	31	106	<6.5
		Fortuna	22	80	<6.5
		Shortana	43	117	8.2
		Era	24	90	<6.5
		Sheridan	30	100	<6.5
Manhattan, Kans.					
Alluvium (limestone)	5.5–6.0	Gage	7	261	<6.5
		Triumph 64	7	198	<6.5
		Kow	4	236	<6.5
		Scout	3	248	<6.5
		Parker	5	208	<6.5
St. Paul, Minn.					
Waukegan sil	6.1	Polk	10	139	11.6
		Chris	19	155	<6.5
		Waldron	13	96	<6.5
		Era	8	158	<6.5
Denton, Tex.					
San Saba c	7.5	Sturdy	30	158	8.6
Udic Pellusterts		Caddo	30	135	7.5
		Fox	27	113	10.5
		Coker-68-15	12	159	<6.5
		Riley-67	14	157	9.8
Temple, Tex.					
Heiden c	7.5–8.0	Sturdy	17	185	<6.5
Udic Chromusterts		Caddo	12	183	<6.5
		Fox	19	118	<6.5
		Coker-68-15	10	256	<6.5
		Riley-67	22	209	<6.5
Bushland, Tex.					
Pullman sicl	7.0	Sturdy	12	176	16.2
Torrertic Paleustolls		Caddo	16	273	9.4
		Tascosa	19	201	10.9
Quinn, S.D.					
Soil developed from Pierre shale	Free $CaCO_3$	Sheridan	38	242	19.0
Bison, S.D.					
Morton sil	Free $CaCO_3$	Sheridan	27	349	<6.5
		Gage	23	152	<6.5
Wall, S.D.					
Ralph cl	Free $CaCO_3$	Gage	18	177	<6.5
Martin, S.D.					
Keith sicl	Free $CaCO_3$	Scout 66	36	189	<6.5
Highmore, S.D.					
Glenham 1	Free $CaCO_3$	Scout 66	30	183	<6.5
Presho, S.D.					
Promise c	Free $CaCO_3$	Scout 66	36	304	20.0
Soil developed from Pierre shale					

[a] Wheat from Montana and Minnesota plus the Sheridan variety from South Dakota are hard red spring wheat varieties. All others are hard red winter wheat varieties. [b] Mean value of three determinations. [c] Mean value of two determinations.

the concentration of Ni in the tobacco plant was directly related to the amount of Ni in solution (Figure 1). Various researchers (Mitchell, 1971; Vanselow, 1966) have reported that Ni extracted from soils correlates highly with plant Ni concentration. However, the data in Table IV suggest varietal differences in the Ni concentration in wheat seeds. Thus, varietal differences in plants must be accounted for before geochemical factors affecting Ni concentrations in plants can be identified.

The small amount of Cr translocated to the aerial portion of plants may not be adequate to meet animal nutritional requirements (Mertz, 1969; Huffman, 1973; Huffman and Allaway, 1973). However, total Cr analyses will allow mapping of geographic areas where plants are relatively higher in Cr than other areas. Although the range in total Cr found in wheat seed (Table IV) was small, it may be nutritionally important (Toepfer et al., 1973).

The data reported here show that different soils support plants containing different concentrations of these three elements. Thus, mapping geographic areas with low and high concentrations of these elements in plants is probable.

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Organic Acids from Fresh California Strawberries

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The acids present in fresh California strawberries were isolated by solvent extraction. Analysis was by coupled gas chromatography-mass spectrometry of the methyl ester derivatives. A total of 33 acids were identified.

The organic acid content of fruit and changes occurring during ripening have been extensively studied (Hulme, 1971). Most of this work has been concerned with the nonvolatile acids. A number of investigators have studied the nonvolatile acids of strawberry (Hulme and Wooltorton, 1958; Johnston and Hammill, 1968; Sistrunk and Cash, 1973). Studies on the aroma constituents of strawberries, however, have revealed a number of volatile acids. These are presented in Table I. In addition, various authors have reported many of the esters of the more commonly occurring acids. The present work describes the isolation and characterization of the more volatile acids from fresh California strawberries. We are using the term "fresh" here only to indicate that the berries had not been frozen. It should be kept in mind that the elapsed time between harvest and analysis was probably about 10 days.

Table I. Volatile Acids Previously Identified in Strawberry

Acetic[a,b]	Tetradecenoic[c]
Butyric[b]	Pentadecanoic[c]
Isobutyric[b]	Palmitic[c]
Valeric[b]	Palmitoleic[c]
Hexanoic[a]	Heptadecanoic[c]
Pentenoic[c]	Stearic[c]
2-Hexenoic[c]	Oleic[c]
Octenoic[c]	Linoleic[c]
Nonanoic[c]	Linolenic[c]
Decenoic[c]	Nonadecenoic[c]
Dodecanoic[c]	Eicosanoic[c]
Tridecanoic[c]	Benzoic[c]
Myristic[c]	Cinnamic[c]

[a] Coppens and Hoejenbos (1939). [b] Dimick and Corse (1958). [c] Tressl et al. (1969).

EXPERIMENTAL SECTION

Extraction and Isolation of Acid Fraction. Two crates (24 pints) of fresh strawberries were obtained from California via National Produce (Long Branch, N.J.). The berries were of the Tioga variety and were harvested in July in Watsonville, Calif. Their organoleptic and visual qualities were good. The strawberries were stemmed, and each pint was ground in a glass Waring Blendor for 15 sec with 400 ml of distilled water. The slurry obtained from each pint was transferred to a 2500-ml glass funnel fitted with a coarse (145-175 μ) fritted disk (Ace Glass, Vineland, N.J.) and allowed to stand until the seeds and other particulate matter had risen to the top. The slurry was then filtered under nitrogen pressure. After the slurry from each pint was filtered, the funnel was back-flushed by forcing nitrogen up through the disk. The combined filtrates (about 8800 ml) were extracted in a separatory funnel in several portions. The initial extraction was done with a total of approximately 900 ml of distilled diethyl ether containing 10% methanol. The emulsion which formed was broken by filtration through absorbent cotton. The two succeeding extractions with about 900 ml each of diethyl ether were carried out with virtually no emulsion formation. The extract possessed a good, fresh strawberry aroma.

The acids were isolated by extraction of the ether phase with three 150-ml volumes of 5% sodium carbonate. The carbonate extract was acidified with 2 *N* hydrochloric acid and back extracted with approximately 300 ml of diethyl ether. The extract was dried over anhydrous sodium sulfate and concentrated by careful distillation in a Kuderna Danish concentrator (Kontes Glass Co., Vineland, N.J.). The last trace of solvent was driven off by a stream of nitrogen.

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