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Nitrogen decontamination by molybdenum in high incidence area of esophageal cancer

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Abstract — Molybdenum (Mo) deficiency and nitrogen contamination have been proved to be relevant to the higher incidence of esophageal cancer. In order to reduce the amount of nitrate and nitrite in grains and vegetables, ammonium molybdate has been used as a trace–element fertilizer in Linxian County, a high risk area of esophageal cancer. This supplementation has significantly raised the yield and the Mo content of the crops. The nitrate and nitrite levels in crops and vegetables were reduced by 12–53 and 20–32% respectively. The amount of ascorbic acid in vegetables which can block the in vivo synthesis of nitroso compounds is increased by 10–70%.

Taking the inhibitory effect of Mo on cancer into account, Mo supplement by using Mo fertilizer probably can serve as an effective chemoprevention measure against this disease in high risk areas.

Keywords: Molybdenum; esophageal cancer; nitrate; nitrite; ascorbic acid.

In contrast to most Western countries, the cancer of esophagus is a common disease in many areas of China, especially in the North. Nitrosamines are the primary suspects for inducing the cancer of esophagus. They can be synthesized either in vitro or in vivo from secondary amines and nitrites. Nitrites usually are originated from nitrates in environment. So it is very important to protect the environment from nitrates and nitrites contamination to reduce their contents in food from high incidence area of esophageal cancer.

Food and water samples from the high esophageal cancer incidence area and its surrounding with medium and low – incidence in Henan Province, have been collected and analyzed (Luo Xianmao, 1980). Data obtained from 909 cereal samples and 686 drinking water samples demonstrated an inverse relationship between the mortality due to this cancer and the content of molybdenum (Mo). Levels of Mo in the serum, hair, and urine of the inhibitants in the high risk area were lower than those in the low risk areas (Cancer Inst. CAMS, 1987). The function of Mo in the reduction of nitrates was fully recognized when the element was shown to be an important constituent of nitrate reductase in plants and nitrate accumulation has been found in Mo–deficient plants (Shkolnik, 1984). The present study was designed to develop an effective measure to reduce the nitrate and nitrite contents in cereals and vegetables from Linxian County, a high risk area of esophageal cancer.

MATERIALS AND METHODS

Sample preparation

Cereal samples were washed with deionized water, dried at 60°C for 18 hrs and stored in a desicator. For the determination of nitrate, nitrite, ammonium and protein, samples were

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ground to a fine powder and then thoroughly mixed. Fresh vegetables were used for detection of ascorbic acid, nitrate and nitrite immediately after collection.

Trace element analysis

Duplicates of samples were wet–ashed in nitric and perchloric acid (5:1 by volume). After dilution, the Mo content in samples was estimated by a catalytic polarography procedure (Cancer Inst. CAMS, 1978) and Cu, Fe, Mg, Mn and Zn by atomic absorption spectrophotometry (Perkin–Elmer 403 AAS).

Protein analysis

The samples were analysed for nitrogen content by a micro Kjeldal procedure. The factor N x 6.25 was used to estimate the protein content (%).

Nitrogen metabolites analysis

Determination of ammonium and nitrate in cereal was made by the method of Black (Black, 1965). Nitrite in cereal and nitrate and nitrite in vegetables were analysed by Hamilton's method (Hamilton, 1976).

Ascorbic acid analysis

Ascorbic acid in vegetables was estimated by method of Suder (Suder, 1952).

Molybdenum fertilization

Ammonium molybdate was used as Mo fertilizer by two methods: foliar spray and seed dressing.

RESULTS

The diet for people in Linxian is extremely simple, consisting mainly of corn, wheat, millet, rice, sweet potato and some seasonal vegetables. The consumption of vegetables and fruits is very low. Nitrate contents in grains, especially in corn and rice, have been decreased significantly after Mo fertilization (Table 1).

Table 1 Effect of Mo fertilizer on nitrate content in cereals

Cereal	No. of tests	Nitrate Control	$M \pm SD$, ppm Mo	Decrease, %	P
Wheat	19	17.1 ± 4.2	13.3 ± 3.5	22.2	< 0.005
Corn	23	6.6 ± 3.4	3.1 ± 2.3	53.0	< 0.001
Millet	19	6.3 ± 2.9	4.7± 2.5	25.4	< 0.001
Rice	12	6.9 ± 3.8	3.6 ± 4.0	47.8	< 0.005

Table 2 Effect of Mo fertilizer on nitrate content in millet

Method	Ammonium molybdate, g/kg seed	Nitrate, ppm	±%
	0	8.4	
Seed	2	6.6	-21.4
dressing	4	5.6	-33.3
	6	9.3	+10.7
	%		
	0	4.4	
Foliar	0.02	2.7	-38.6
spray	0.05	1.3	-70.5
	0.20	4.4	0
	0.50	8.0	+8.16

The reduction of nitrate content in rains was dependent on the dose of ammonium molybdate used. Excess fertilization not only raised the nitrates levels in grains (Table 2), but also decreased the output of wheat in Linxian (Table 3).

Mo markedly increased ammonium content in wheat ($P < 0.05$), but no significant change was observed in other grains (Table 4). No detectable nitrite was found in both control and fertilized grains (detection limit = 0.01 ppm).

Table 3 Effect of Mo treatment on the yield of wheat

Ammonium molybdate, g/kg seed	No. of tests	No. of Tests with the yield Increase	Decrease	Average increase in the yield, %
1	43	43	0	14.4
2	38	37	1	15.5
3	33	27	6	11.5
4	22	14	8	3.1
5	18	9	9	1.4

Table 4 Effect of Mo fertilizer on ammonium content in cereals

Cereal	No. of tests	Ammonium $M \pm SD$, ppm		±%	P
		Control	Mo		
Wheat	19	17.4 ± 7.1	21.2 ± 7.4	+21.8	< 0.05
Corn	23	21.4 ± 7.6	23.0 ± 9.8	+7.5	> 0.05
Millet	19	11.1 ± 3.2	10.5 ± 2.5	-5.4	> 0.05
Rice	12	8.4 ± 0.9	8.1 ± 1.7	-3.6 > 0.05	

Table 5 Mo content in cereals after Mo fertilization

Cereal	Mo $M \pm SD$, ppm Control	Mo	Increase, %	P
Wheat	0.47 ± 0.18 (37)	0.85 ± 0.39 (37)	80.9	< 0.001
Corn	0.33 ± 0.08 (26)	0.50 ± 0.13(22)	51.5	< 0.001
Millet	0.37 ± 0.08(22)	1.01 ± 0.93(19)	172.0	< 0.005
Rice	0.40 ± 0.10(17)	0.88 ± 0.77(13)	120.0	< 0.05

* No of tests in parentheses

Table 6 Cu, Fe, Mg, Mn and Zn content in cereals with or without Mo fertilizer

Cereal	Treatment	No. of samples	Cu	Fe	Mg, mg/kg	Mn	Zn
Wheat	Control	7	4.7	46.9	1213	38.4	19.2
	Mo	15	5.1	46.1	1237	38.5	19.6
Corn	Control	10	1.6	23.8	897	5.4	15.8
	Mo	25	1.7	23.9	891	5.5	15.9
Millet	Control	8	5.3	32.6	1052	13.5	19.1
	Mo	19	5.4	31.1	1186	13.8	19.2
Rice	Control	4	1.6	13.3	990	17.6	13.3
	Mo	13	1.7	12.9	987	16.8	12.4

All P values > 0.05

Analysis of 34 wheat and 2 corn samples was performed for protein. Protein content increased only in 2 wheat samples, from 8.5 % in control to 10.6 and 11.5 % respectively, and remained generally unchanged in the others.

Mo fertilizer raised the Mo contents in wheat, corn, millet and rice by 80.9, 51.5, 172.0 and 120.0 % respectively (Table 5), but it didn't influence the levels of Cu, Fe, Mg, Mn and Zn in grains (Table 6).

As to the influence of Mo fertilizer on vegetables, Table 7 shows that nitrate and nitrite contents in Mo–fertilized vegetables were decreased by 12.0 – 22.1 % and 20.9 – 32.6 % respectively, and ascorbic acid level was raised by 10.9 – 70.4 % in comparison with control.

The economic benefits from Mo application to agriculture in Linxian County can be seen from Table 8.

Table 7 Vitamin C, nitrate and nitrite contents in vegetables with or without Mo fertilizer

		Vitamin C		Nitrate,		Nitrite	
Vegetable	Treatment	mg/100g	Increase,%	ppm	Decrease,%	ppm	Decrease, %
Greens	Control	196		20.0		0.67	
	Mo	334	70.4	16.0	20.0	0.53	20.9
White	Control	26.6		8.3		0.43	
radish	Mo	29.8	12.0	7.3	12.0	0.29	32.6
Chinese	Control	201		14.9		0.56	
cabbage	Mo	223	10.9	11.6	22.1	0.40	28.6

Table 8 Increase of cereal production by Mo fertilization in Linxian

Cereal	No. of tests	Average increase, %
Wheat	114	13.8
Corn	28	18.9
Millet	15	20.8
Rice	4	8.6

DISCUSSION

The nitrogen contamination in the environment is a cause of concern since the availability of nitrate and nitrite from the food and water supply may favor the in vivo synthesis of N–nitroso compounds, for which experimental evidence of their mutagenic and carcinogenic activities has been reported (Sugimure, 1970; Endo, 1973). Earlier publications presented presumptive, epidemilogic evidence for nitrogen contamination as a factor in the etiology of stomach cancer (Cuello, 1976; Fraser, 1980). The average concentration of nitrates and nitrites in the water of most wells in the 49 villages of Linxian County showed a positive correlation with the average incidence rates of esophageal cancer and marked epithelial hyperplasia, a precancerous lesion of the esophagus, for the years 1969–1976 (Wang, 1979). Nitrates and nitrites were detected in human gastric juice and saliva in Linxian. The salivary nitrites were significantly higher in patients with marked epithelial dysplasia or carcinoma of the esophagus than in the normal controls (Cancer Inst. CAMS, 1978).

The relationship between Mo deficiency and the incidence of esophageal cancer in humans was first reported by Burrel (Burrel, 1966). The high incidence of this cancer among the Bantu of Transkei in South Africa was attributed to the consumption of food locally grown in soil low in Mo. Nemeko *et al.* also have reported that the Mo content in the drinking water was lower in the high–incidence area of esophageal cancer than in the low–incidence areas in Russia (Nemeko, 1976). But no further investigation in this respect was done in both such areas.

Our previous studies provided the evidence that Linxian is a low Mo area. On average, the self–selected diets in this area afford only 65.4 ± 11.4μg Mo/day for men (our unpublished data), which is much less than 150 μg, the minimum value of Recommended Dietary Allowance (RDA) for Mo (Food and Nutrition Board, U.S. National Research Council, 1980). The Mo deficiency in Linxian is also evidenced indirectly by the successful application of Mo fertilizer to agriculture in this area (Table 8).

Mo deficiency in plants is not direct but as a function of the supply of nitrogen to plants to be closely connected with the assimilation and reduction of nitrates. In Mo – deficient area, nitrates are usually accumulated in crops. In order to reduce the amount of nitrate and nitrite in grains and vegetables, ammonium molybdate has been used as a trace–element fertilizer in Linxian. The results from our experiment have shown that Mo not only increased the output of grains, but also significantly reduced the contents of nitrates and nitrites, the precursors of N–nitroso compounds in grains and vegetables. But the marked decrease in nitrate and nitrite contents was not observed in all fertilized grains and vegetables. There may be several factors involved:

1. The requirement of plants for Mo is usually much more strongly manifested in the case of nitrate as the sole nitrogen source. If ammonium or nitrite nitrogen was supplied, the effect of Mo was usually only slight (Shkolnik, 1984).

2. Excess Mo fertilizer may result in nitrate accumulation in plants as shown in Table 2. But the mechanism of diphasic effect of Mo on nitrate assimilation in plants remains obscure.

3. Nitrate accumulation has been found in the cases of other trace elements, for example, iron and manganese. For the purpose of reducing nitrate intake of inhabitants in Linxian, it is desirable to apply Mo fertilizer reasonably and to study the effect of iron, manganese and other trace elements on crops and vegetables.

In plants, the most important aspect of the physiology of Mo is its involvement in nitrogen metabolism, particularly in the reduction of nitrates and the fixation of molecular nitrogen. The assimilation of nitrate by plants is a process whereby oxidized inorganic nitrogen is reduced to assimilatable ammonia, that is achieved by the concerted efforts of only two enzymes: nitrate reductase, which catalyzes the reduction of nitrate to nitrite, and nitrite reductase, which catalyzes the reduction of nitrite to ammonia (Adams, 1985). The latter is utilized in the subsequent synthesis of proteins. In our experiments, no detectable nitrite was found in grains either with or without Mo fertilization. It implied probably that nitrites were only the shortlived intermediates in process of nitrate assimilation. It seems not necessary to be concerned in nitrite accumulation in grains after Mo fertilization.

Our data indicate that Mo can raise the yield of main crops in Linxian, but no increase in protein percentage has been found in most Mo–fertilized grains.

Nitrogen metabolism and protein synthesis in plants are very complicated processes, in which a lot of enzymes are involved. Wheat may have a different metabolic pattern of nitrates from other grains, that results in a significant raise of the ammonium content in seeds after Mo fertilization.

Mo also influences the metabolism of vitamins in plants. A number of studies show that there is a dramatic fall in ascorbic acid content in Mo–deficient plants. This fact now is taken as a specific sign of Mo deficiency (Hewitt, 1950; Burkin, 1968). Our experiments that Mo supplement could markedly increase ascorbic acid content in vegetables have verified their observations. The possibility that Mo is involved in the biosynthesis of ascorbic acid could not be ruled out. It is well known that ascorbic acid can block the in vivo synthesis of N–nitroso compounds.

Utilizing animal models, we have substantiated, for the first time, the epidemiological implication of Mo deficiency on carcinogenesis. Our earlier studies demonstrated that Mo supplementation could significantly inhibit N–nitrososarcosine ethyl ester–induced esophageal and forestomach carcinogenesis in rats and mice (Luo Xianmao, 1983; Wei Huijuan, 1986). It is likely that Mo supplementation by using Mo fertilizer can serve as an effective, chemoprevention measure against esophageal cancer in high incidence areas. The safety of this measure can be guaranteed by the following: 1. The adequate Mo content in grains was always maintained when the dose of Mo fertilizer was optimum; 2. The Mo intake calculated from Mo–fertilized food in Linxian is only close to the minimum value of recommended safe and adequate range; 3. No marked change was found in the contents of other elements in Mo–fertilized grains (Table 7).

Esophageal cancer is a multistage and multifactor disease and its time course commonly extends over 20 years or more. Although a great deal of information on it has been obtained, the causative factors of this cancer remain to be established. Therefore it is desirable to apply Mo fertilizer in combination with other preventive measures to reduce the incidence of esophageal cancer.

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