Manganese Contents in Fruits and Soils in Elazig-Turkey Region

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Summary: Manganese concentrations in fruit samples were determined by using flame atomic absorption spectrometry (FAAS). To identify the Mn phases most responsible for fruit-available Mn, the soil samples near the fruit plants were also analyzed for Mn by using various digestion and selective extraction reagents. The relation between the fruit-Mn levels and the Mn concentrations in soil extracts was studied. Total Mn concentrations determined in the studied soils and fruits were found in the range of 330 to 1100 and 1.6 to 22 mg/kg, respectively. The negative interferences were observed from Ca, Mg and Al at the higher concentrations than the their concentrations at the measurement step. It was observed that the Mn concentrations of mulberry samples correlate to the manganese concentrations of the cold citric acid extracts of soils

Introduction

The levels of trace elements in food and agricultural samples have been shown to influence human and plant metabolism [1]. All Mn compounds are very important soil constituents because this element is essential in plant nutrition and controls the behavior of several other micronutrients [2]. On the other hand, Mn not been considered to be a polluting metal in soil. Maximum allowable concentration (MAC) of Mn in agricultural soils is estimated at 1500 mg/kg. All plants have a specific requirement for manganese and apparently the most important of Mn functions is related to the oxidation-reduction process. Mn^{+3} is known to be a specific component of two enzymes, arginase and phosphotransferase. Therefore, adequate level of available Mn is necessary in plant nutrition. The deficiency symptoms of manganese occur firstly in younger leaves as interveinal chlorosis, necrotic spots on leaves and reducing of turgor. The most sensitive plants are cereals, legumes and fruit trees such as apples, cherries and citrus [2].

Mn is likely to occur in soils as oxides and hydroxides in the form of coatings on soil particles as nodules of different diameter. But the solubility of soil Mn is of significance since the plant supply of Mn depends mainly on the soluble Mn pool in the soil. The most common symptom of Mn toxicity is Fe chlorosis. Mn-Fe antagonism is widely known and these binaries are interrelated in their metabolic functions.

The speciation of Mn in soil is more important for estimating its uptake by plants and the food chain because the amount of the transferred manganese to these matrices depend on the chemical species of Mn than its total amount in soil. In one broad definition of speciation [3], the species are defined by their function, as, for example, 'plant available forms', 'exchangeable cations' or Labile species'. The general approaches has been to separate the soil into different chemical or physical fractions and, by analysing each fraction, to determine the amount of element, combined or associated with each soil fraction or phase. Alternatively the fractionation is performed by extraction of the soil with chemical reagents or solvents designed to extract the elements bound in, or associated with, a particular soil phase or component.

A number of extractants, including ethylene-diaminetetraacetic acid (EDTA), diethylenetriamine pentaaceticacid (DTPA), acetic acid, ammonium acetate, calcium chloride and NH_2OH. HCl have been tested to identify metal species as exchangeable, carbonate-bound, Fe and Mn oxide-bound, organically bound, and to estimating the plant or fruit available trace metals [4-9].

Flame atomic absorption spectrometry (FAAS) has been proved to be reliable and convenient method for the metal analysis in food, biological, and environmental matrices as direct or, if

necessary, in combination with preconcentration methods [10-16].

In this study, Mn concentrations in the fruit and soil samples were determined by FAAS. The possible chemical forms of manganese in soil were examined by using various selective extraction reagents such as oxalic acid, citric acid, Na_2EDTA, acetic acid and a nitric acid/hydrogen peroxide mixture. The relation between the manganese contents of fruit samples and the manganese contents of soil extracts was investigated.

Results and Discussion

In the literature, the values between the dry ashing and wet ashing methods for Mn determination in vegetables showed no statistically significant differences [13]. Therefore, dry ashing method was preferred because this method is simple and smaller quantities of reagents were needed which reduces the possibility of extraneous pollution. It is seen that from Fig 1-3, the major components up to described concentrations as follow: (mgL^{-1}) Ca:400; Mg:800 and Al:150 were not interfered at the measurement step when the small amounts of samples were taken. These major elements mentioned above have suppressed the Mn absorbance at higher concentrations than the concentrations mentioned above Fig. 1-3). Iron, one of the major elements, was not interfered in the concentration range of 50 to 800 mgL^{-1}. Although these elements were found in soil samples at much high concentrations, Mn is also found at high concentration in the studied soils and therefore, the dilutions were need. Thus, Ca, Mg, Al and Fe are also diluted. For example, total Al concentrations of the studied soils were found in the range of 3800 to 8500 $mgKg^{-1}$ (unpublished data). When 0.25 g of soil was digested and final volume was diluted to 2.0 ml, Al concentration in this solution is existing as maximum 2000 mgL^{-1}. For the Mn determination in these soil samples, at least, 100 fold dilution is need and so, Al concentration in the solution diluted 100 fold is existing 20 mgL^{-1}.

Before the digestion step, the added Mn amounts in its nitrate form to the fruits and soils were recovered to check accuracy of analyses performed. The acceptable recoveries were achieved for the examined fruits and soils (Table 1). It was found that at least 90 % of the Mn added as $Mn(NO_3)_2$ to the fruit samples was recovered. The recoveries obtained for the Mn added as $Mn(NO_3)_2$ to soil matrices were

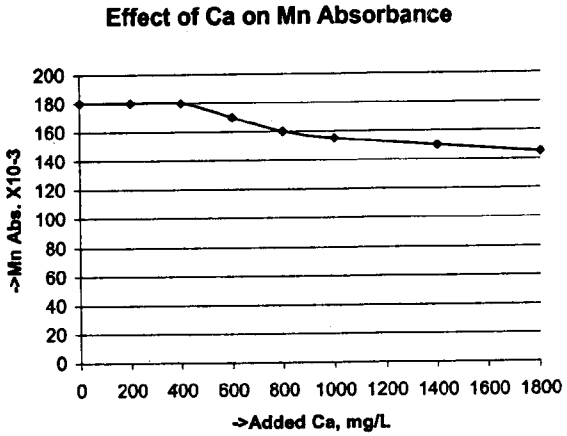

Fig. 1: Effect of added Ca on the absorbance of Mn. Mn: 2 mgL^{-1}

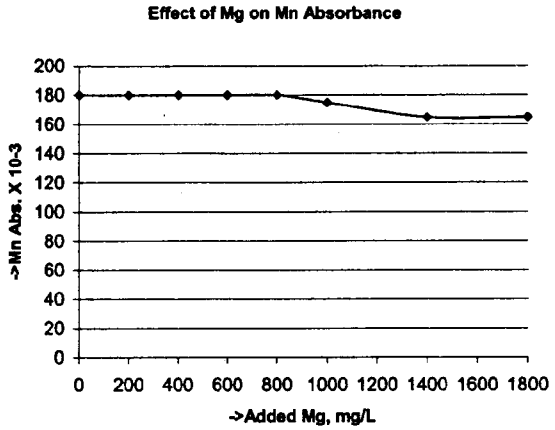

Fig. 2: Effect of added Mg on the absorbance of Mn. Mn: 2 mgL^{-1}

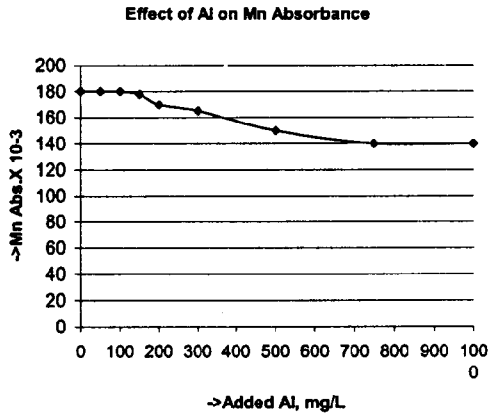

Fig. 3: Effect of added Al on the absorbance of Mn. Mn: 2 mgL^{-1}

Table 1: Recoveries of Mn from fruit and soil by using HNO_3/H_2O_2 mixture (dry weight basis). The results are mean values±standard deviation; n=4

Sample*	Mn levels in Fruit,			Mn levels in Soil		
	Added mg kg^{-1}	Found mg kg^{-1}	Recovery, %	Added mg kg^{-1}	Found mg kg^{-1}	Recovery, %
Morello cherry 2	0	6.5±0.4	-	0	485±30	-
Morello cherry 2	2	8.3±0.5	90	100	580±35	95
Strawberry 1	0	22±1.0	-	0	1100±72	-
Strawberry 1	10	31.5±1.5	95	250	1330±65	92
Apple 7	0	2.4±0.2	-	0	580±33	-
Apple 7	2	4.2±0.10	90	100	673±35	93

• Numbers such as 1, 2 and 7 refers to the region from which soil and fruit samples were taken.

Table 2: Results of Mn contents of fruits and soils (dry weight basis). The results are mean values±standard deviation. n=4, pH±0.2. Mn concentrations in cold acetic acid extracts were found lower than the concentration of 10 $mgkg^{-1}$. Therefore, these values were not given in this Table.

Sample of Fruit and Soil on grown[b]	Mn in Fruit $mgkg^{-1}$	Mn in soil, mg/kg								
		HNO_3/H_2O_2	Oxal. a[a] 1 M	Oxal. a 1 M	EDTA 0.05M[a]	EDTA 0.05M	Ace. acid Concen.[a]	Citric a. 1 M	1.5 M HNO_3	Soil pH
Morello cherry1	4.0±0.2	440±25	300±13	140±10	205±7	125±6	165±6	75±4	105	6.4
Morello cherry2	6.5±0.4	485±30	320±30	110±7	260±9	30±2	310±12	195±9	270	6.3
Morello cherry3	4.8±0.3	360±26	120±10	65±4	160±5	30±2	145±6	130±7	90	6.4
Morello cherry4	5.3±0.3	755±20	650±25	300±18	430±15	340±19	400±13	185±8	230	6.2
Morello cherry5	4.9±0.3	460±33	180±8	110±7	220±6	30±2	200±7	170±8	170	6.1
Cherry 6	2.6±0.2	1000±60	750±12	450±26	655±20	70±3	535±16	150±7	375	6.6
Cherry 4	2.5±0.2	810±42	600±27	310±15	500±21	250±14	410±13	250±11	240	6.3
Mulberry 1	4.5±0.3	450±23	310±11	160±10	220±9	140±9	170±6	80±4	110	6.4
Mulberry 3	5.0±0.2	330±25	105±8	65±5	120±5	35±2	130±5	165±8	80	6.4
Mulberry 4	6.0±0.3	730±21	710±26	350±17	630±18	300±15	380±15	297±16	260	6.4
Mulberry 5	4.8±0.2	470±32	210±10	105±6	230±10	25±2	200±9	148±6	200	6.5
Strawberry 1	22±1.0	1100±72	900±35	450±20	850±32	80±4	480±18	232±13	390	6.5
Strawberry 6	21±0.9	930±65	1000±17	400±19	600±13	70±4	400±15	180±10	330	6.6
Apple 4	1.6±0.1	400±15	300±15	200±10	270±9	150±6	240±8	100±7	120	6.3
Apple 7	2.4±0.2	580±33	480±18	270±12	550±11	80±4	380±14	296±14	315	6.4
Grape 5	4.5±0.2	600±37	320±18	190±17	380±14	50±3	240±9	292±15	320	6.4
Grape 7	5.0±0.3	900±42	900±30	480±14	750±20	100±6	420±17	158±9	350	6.7
Pear 7	2.7±0.2	560±30	320±13	230±10	500±11	40±2	350±15	260±12	300	6.5

[a]: Hot extraction

[b] Numbers such as 1, 2, 3, 4, 5, 6 and 7 refers to the region from which soil and fruit samples were taken.

higher than 92 % by using HNO_3/H_2O_2 digestion. The effect of contamination was eliminated by subtracting values obtained for blanks. Adsorption loss can be excluded as the procedure was followed in exactly the same way, using the same glassware and the same reagents that were used throughout. Therefore, the effect of contamination or adsorption may be reliably overlooked.

Calibration curve was obtained by using the manganese solutions of 0.50; 1.0; 1.5; 2.0 and 4.0 mg L^{-1} containing the matrix components at the concentrations as follows (Ca: 400; Mg: 400; Al: 100 and Fe: 100 mgL^{-1}). The graph obtained was rectilinear in the concentration range of described above and the equation of the curve was as follow:

$Y= 87.118X + 2.7647$ $R^2=0.99$ (R^2:correlation coefficient)

Manganese Contents of Fruits and Soils

Table 2 gives the Mn concentrations of fruit by using dry ashing and the soil samples by using different selective extraction reagents. The mean total manganese concentrations for all studied fruits were in the range of 1.6-22.0 mg kg^{-1} which the lowest level for apple and the highest level for strawberry. In literature, soil EDTA-extractable metal concentrations were found as correlate with the available amounts of metals in plant uptake [2]. Our results show that these evaluations for Mn are not valid for morello cherry and mulberry. Other observations concerning Table 2 are as follows.

The changes in the manganese concentrations of morello cherry does not depend on the Mn contents of all studied extracts of soil, but there are a tendency (Fig 4) towards the correlation between the

morello cherry-Mn and the cold citric acid extracts of soil-Mn (R^2=0.74).

It is seen that from the Fig. 5, the Mn concentrations in mulberry samples did linearly change as dependent on only the Mn concentrations in the cold citric acid extracts of soil (R^2=0.99). In addition, there are a tendency towards the correlation (Fig. 5) between the Mn concentrations of the mulberry and soil-EDTA-extracts (R^2=0.76) and soil-HNO_3/H_2O_2 digestions (R^2=0.64).

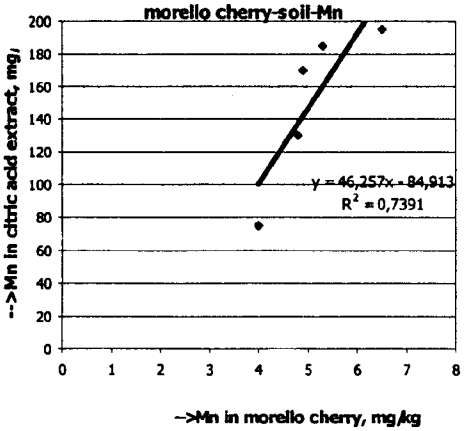

Fig. 4: Relationship between the amounts of morello cherry-Mn and soil-citric acid-extractable-Mn

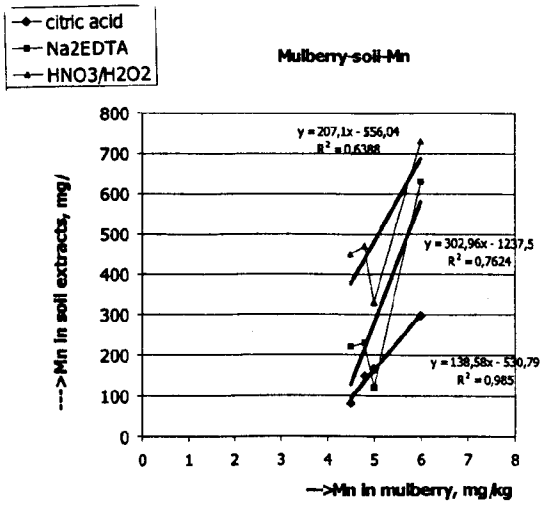

Fig. 5: Relationship between the amounts of mulberry-Mn and soil-Na_2EDTA, soil-HNO_3/ H_2O_2 and soil-citric acid-extractable-Mn.

The Mn content of the cherry and strawberry fruits did change as dependent on the Mn concentrations of all soil extracts except cold Na_2EDTA and citric acid for cherry whereas except cold oxalic acid for strawberry.

Similarly, the Mn content of the apple and grape samples did change as dependent on the Mn concentrations of all soil extracts except cold Na_2EDTA for apple whereas except cold citric acid for grape.

Mn concentrations in the hot oxalic acid extracts of soil were close to the Mn concentrations in the HNO_3/H_2O_2 digestion of soil for all samples of the location 4, 6 and 7 (Table 2). These results can be caused of the Mn species in these samples are both the reducible forms having its oxides and hydroxide forms and occluded on minerals such as Fe oxide. So, oxalic acid plays the role both acid and complexing agent which form complexes with both Fe and Mn and so, manganese releases from minerals (Overall complex formation constant of Fe^{+3}-oxalate: 6.3 10^{45} [17]. On the other hand, Mn concentration in the hot Na_2EDTA extracts of soil were also close to the values of HNO_3/H_2O_2 digestion and the hot oxalic acid extracts for all samples of the location 7. Therefore, it can be said the manganese species in these soils are organically bound. Probably, manganese species in the soil samples of location 6 are present both organically bound and as its carbonate form because Mn concentrations in the hot Na_2EDTA and hot acetic acid extracts of these soils were considerably high and very close to each other.

The comparative data obtained by using various selective extraction reagents were found useful to fractionation of manganese in soils and to estimate the Mn uptake of fruits from soils. The results obtained show that the Mn contents of fruits are dependent to the different extractants-soil Mn concentrations as related to the fruit types.

Experimental

Apparatus and Reagents

An ATI UNICAM 929 Model flame atomic absorption spectrophotometer (AAS) equipped with ATI UNICAM Hollow cathode lamp was used for the Mn determination. The optimum conditions for FAAS were as follows: wavelength; 279.5 nm, HCL current; 11.5 mA, the flow rate of acetylene and air;

1.0 and 4.0 L/min, respectively and slit width; 0.2 nm.

Unless stated otherwise, all chemicals used were of analytical-reagent grade. Throughout all analytical work, doubly distilled water was used. All glass apparatus have been kept permanently full of 1 M nitric acid when not in use. In the digestion and extraction procedures, concentrated nitric acid (65%, Merck-00456), hydrogen peroxide (35%, Merck), oxalic acid (Merck), citric acid (Merck), ethylenediaminetetraacetic acid disodium salt (Na_2EDTA, Merck) and acetic acid (96%, Merck-00062) were used. Mn concentrations in the concentrated nitric and acetic acids were lower than 0.01 mgL^{-1}. Stock solution of Mn (1000 mg L^{-1}) was prepared by dissolving $Mn(NO_3)_2 .4H_2O$ in 1.0 mol L^{-1} nitric acid.

Preparation of Samples

Seven sites were selected from the major agricultural areas in Elazig, Turkey. The soil samples were taken from these seven different locations, with the sampling at a depth of about 10 cm below the surface. The fruit samples were also gathered at each of these locations, at distances not more than 100 cm from the location of the soil sample because the soil samples collected are representative of the rooting environment of the plant at these distances. Morello cherry, Cherry, Mulberry, Strawberry, Apple, Grape and Pear were chosen for the present study because they are the common consumed fruit available in Turkey. The fruit samples were collected in plastic bags. The fruits chosen were washed separately and thoroughly with running tap water and further rinsed twice with distilled water and then allowed draining on a filter paper. The seed of Morello cherry, Cherry, Apple and Pear were removed. The Mulberry, Strawberry and Grape were used as whole fruit. All studied fruits were used without stalk and green leaves and together with skin. The pear and apple samples were cut into small pieces for easy drying. Then, both fruit and soil samples were dried at 85 °C. The time for drying were in the range of 24-48 h dependent on the fruit type. The ratio of dry samples to the fresh samples were found as percentage for morello cherry:14; mulberry:17; cherry:16; strawberry: 15; apple: 17; grape:15; and pear:15

Dry Ashing of Fruits

After grinding and homogenising for better sampling, approximately, 0.5-1.0 g samples of oven dried materials were placed into evaporating dishes and ashed at 480 °C in an ashing furnace for 4 h. This process was repeated if necessary until a white ash was obtained. The mixtures of nitric acid-hydrogen peroxide (2+1) (for 0.5 g of dried matter, 1.5 ml of mixture were used) were added to the ashed samples and dried with occasionally stirring on a hot plate with low heat. Then, the residue was dissolved with 3.0 ml of 1.5 mol L^{-1} nitric acid and, if necessary, diluted to suitable volume. After centrifugation, the clear digests were analysed for Mn by FAAS. A blank digest was carried out in the same way.

Digestion and Extraction of the Soil

Soil pH was measured after filtration of the soil suspension, using soil: distilled water at 1:5 (w/v). The mixture of nitric acid-hydrogen peroxide (2+1) of 1.5 ml were added to the soil samples of 0.25 g and dried with occasionally shaking on a hot plate. After cooling, 2 ml of 1.5 mol L^{-1} nitric acid was added to the remainder and centrifuged. After necessary dilution, the clear digests were analysed by using FAAS. A blank digest was carried out in the same way.

The soil extracts were obtained by using two extraction procedure. a) cold extraction: one ml of 0.05 mol L^{-1} Na_2EDTA (for carbonate and organically bounded phases), 1.0 mol L^{-1} oxalic acid (for carbonate and Fe and Mn oxide phases), concentrated acetic acid (for carbonate phases) and 1.0 mol L^{-1} citric acid were separately added to 0.25 g of different portions of each one of soil samples at the room temperature and stirred by vortex for 5 min (this procedure was mentioned as cold extraction in this text). b) hot extraction: one ml of 0.05 mol L^{-1} Na_2EDTA, 1.0 mol L^{-1} oxalic acid and concentrated acetic acid was separately added to 0.25 g of different portions of each one of soil samples. The mixture was evaporated with occasionally shaking on a hot plate. After cooling, 2 ml of 1.5 mol L^{-1} nitric acid was added and centrifuged (this procedure was mentioned as hot extraction in this text). In addition, 2 ml of nitric acid solution of 1.5 mol L^{-1} was added to each one of the soil samples for the identification of the nitric acid effect on Mn extraction at the hot extraction procedure. After centrifuging, all solutions were diluted to suitable volumes. The clear digests were analysed for Mn by using FAAS. The blank digests were carried out in the same ways.

References

1. Essential and Toxic Trace Elements in Human Health and Disease, A.S. Prased, Ed. (Alan R. Liss), New York (1988).
2. A.K. Pendias and H. Pendias, in:"Trace Elements in Soils and Plants", CRC press Inc., Florida, Third ed. (2000)
3. A. M. Ure, *Mikrochim. Acta (Wien).* **II**, 49 (1991)
4. V. Spevackova and J. Kucera, *Intern. J. Environ. Anal. Chem.*, **35**, 241(1989)
5. M. Verloo and M. Eeckhout, *Intern. J. Environ. Anal. Chem.*, **39**, 179 (1990).
6. M. Yaman, Y. Dilgin, and S. Gucer, *Anal. Chim. Acta,* **410**(1-2), 119(2000).
7. M. Yaman, *Bull. of Environ. Contam. and Toxicol.*, **65**(4), 545 (2000)
8. M. Yaman, *Comm. Soil Sci. Plant Anal.*, **31**(19-20), 3205 (2000)
9. M. Yaman, *J. Anal. Chem.*, **56**(5), 417 (2001)
10. V.D. Zheljazkov and N.E. Nielsen, *Plant and Soil,* **178**, 59 (1996).
11. P.J.Oles and W.M. Graham, *J. Assoc. Off. Anal. Chem.*, **74**, 812 (1991).
12. Z. Benzo, H. Schorin and M. Velosa, *J. of Food Science*, **51**(1), 222 (1986)
13. M. Yaman, *Chem. Anal. (Warsaw),* **42**, 79 (1997)
14. M. Yaman, *Mikrochim. Acta*, **129**, 115 (1998)
15. M. Yaman, *J. Anal. At. Spectrom.*, **14**, 275 (1999)
16. M. Yaman and S. Bakirdere, *Mikrochimica Acta*, **141**, 47 (2003).
17. J.A. Dean, ed., Lange's handbook of Chemistry, Twelfth ed., McGraw-Hill, New York (1979)