# MANGANESE DETERMINATION IN HAIR SAMPLES

by

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## Manganese determination in hair samples

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### Introduction

In nutritional studies of cattle opinions differ as to the necessity of manganese. But in general it is believed that manganese is necessary for the health of cattle and thus it is important to evaluate a method for the manganese status of cattle. It is known that the estimation of the manganese content of the hair samples from cattle may be an useful guide for the manganese status of the animal. The present investigation was made for testing the reliability of the determination of manganese in hair. Besides, attention has been paid to the way of cleaning the hair sample and the differences in manganese content of white and coloured hairs.

In the Netherlands the method described by Van Koetsveld (1958) is used for hair analysis. In this method the sample is ashed at 500°C till the ash is white to yellow-white. The manganese is then dissolved in dilute acid and oxidised to permanganate. The colour intensity is measured in a spectrophotometer.

Oosting (1956) has drawn special attention to the ashing of organic material in relation to the determination of trace elements. He has pointed out the drawbacks of dry ashing and the most important point to reckon with is the possibility of losses by formation of volatile organo-metallic compounds. Besides these drawbacks the method of dry-ashing suffers from another defect. It has been often observed that the outer surface of the ashed hair samples presents a yellow or white colour, while the inner part of the sample remains incompletely ashed. As a result, low values are then obtained. The explanation is probably that the incompletely ashed material reduces partially the permanganate formed in the reaction. Though this can be prevented by ashing during two days, yet it is a disadvantage. Further it is observed, that after dissolving the residue, the solution sometimes appears to be yellowish which may also cause to high values in the optical density measurement.

Kniphorst (1946) warns against the use of perchloric acid in the wet digestion for manganese determination. In agreement with Kahane and Brand (1934) he points out that there may be substantial losses by formation of volatile chlorides caused by the use of this acid. Oosting, however, digested organic materials with a mixture of sulfuric acid and perchloric acid and found no losses. We used a mixture of nitric acid, sulphuric acid and perchloric acid in the ratio 10 : 1 : 1 and in agreement

### Experimental

The method of estimation has been studied from the following points of view:

with Oosting did not find any loss due to the presence of perchloric acid.

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I. Comparison of wet digestion and dry ashing.
II. Comparison of the permanganate with the formaldoximemethod.
III. Way of cleaning of the hair sample before digestion.
IV. Manganese content of coloured and white hairs in connection with the sampling.

## Results

### Ia. COMPARISON OF DIFFERENT MANGANESE SOLUTIONS DETERMINED AS PERMANGANATE (CALIBRATION CURVES)

Three calibration curves have been compared. These curves have been made:

1. by direct measurement of permanganate solutions of known strength;
2. by dry ashing at 500°C for 48 hours of known quantities of manganous sulphate and measuring according to the permanganate method of Van Koetsveld;
3. by wet digestion with the above mentioned mixture of $HNO_3$ (1,4), $H_2SO_4$ (1,84) and $HClO_4$ (1,67) in the ratio 10 : 1 : 1, of known quantities of manganous sulphate and measurement according to the permanganate method.

The extinctions measured at 530 nm in a cell of 10 mm pathlength in a Beckman spectrophotometer DU (G 2400) are mentioned in table 1. The results obey the law of Beer. The calibration factor ($\frac{\text{conc. } \mu g \text{ Mn}}{\text{extinction}}$, in which concentration means $\mu$g per ml) is in column A, B and C respectively 23,9, 23,6 and 23,0. The differences are small.

*Table 1*

*Extinctions corrected for the blank at different treatments of known quantities of manganese measured according to the permanganate method.*

| $\mu$g Mn in 50 ml solution | A permanganate solution directly measured | B dry ashing of manganous sulphate | C wet ashing of manganous sulphate |
|---|---|---|---|
| 10 | 0,008 | — | — |
| 25 | 0,020 | 0,023 | 0,023 |
| 50 | 0,042 | 0,045 | 0,045 |
| 75 | 0,063 | 0,066 | 0,065 |
| 100 | 0,082 | 0,086 | 0,087 |
| 125 | 0,104 | 0,108 | 0,112 |
| 150 | 0,126 | 0,123 | 0,127 |
| 175 | 0,147 | 0,146 | 0,152 |
| 200 | 0,168 | 0,166 | 0,173 |

### Ib. COMPARISON OF DRY AND WET ASHING OF HAIR SAMPLES

In the introduction it has been mentioned that incomplete ashing of hair samples may be a drawback of the dry ashing procedure. This drawback is, however, obviated if the sample is ashed for 48 hours. For this reason

we have made a comparison between dry ashing for 48 hours and wet digestion with the 10 : 1 : 1 acid mixture. The permanganate method has been used for the determination of the manganese.
In table 2 the results of duplicate determinations with both methods of 18 samples are given.

*Table 2*

*Reproducibility of manganese analyses of hair samples in dry and wet ashing as determined with the permanganate method.*

| hair sample | | manganese content p.p.m. | | | |
|---|---|---|---|---|---|
| | | dry ashing | | wet digestion | |
| nr. | colour | $x_1$ | $x_2$ | $x_1$ | $x_2$ |
| 1 | black | 9,5 | 5,6 | 7,7 | 4,8 |
| 2 | „ | 16,7 | 16,7 | 14,5 | 14,1 |
| 3 | „ | 14,7 | 12,3 | 10,0 | 10,0 |
| 4 | „ | 13,1 | 14,3 | 11,9 | 11,9 |
| 5 | „ | 9,5 | 9,1 | 6,3 | 6,3 |
| 6 | „ | 10,7 | 10,7 | 8,2 | 8,2 |
| 7 | „ | 15,1 | 21,0 | 18,5 | 17,9 |
| 8 | „ | 8,7 | 11,5 | 10,7 | 10,7 |
| 9 | „ | 6,3 | 4,0 | 7,1 | 7,1 |
| 12 | white | 2,8 | 13,1 | 7,7 | 4,8 |
| 13 | „ | 2,4 | 2,4 | 2,2 | 2,2 |
| 14 | red | 40,1 | 22,6 | 24,9 | 23,1 |
| 16 | „ | 19,8 | 25,8 | 14,9 | 14,9 |
| 17 | „ | 52,8 | 38,0 | 26,2 | 24,4 |
| 18 | „ | 25,8 | 32,9 | 23,4 | 24,9 |
| 19 | „ | 43,7 | 50,0 | 28,0 | 30,4 |
| 20 | „ | 26,2 | 16,3 | 14,9 | 13,4 |
| 21 | „ | 42,1 | 34,1 | 30,1 | 29,8 |

The difference between the replicates in dry ashing is rather large. With the formula $s = \sqrt{\frac{\Sigma\, d^2}{2n}}$, in which d is the difference between two replicates and n is the number of samples, the standard deviation for an individual estimate has been calculated. The standard deviation for a single analysis of dry ashing is higher ($s\% = 26.99$) than in wet digestion ($s\% = 6.67$). So the reproducibility for wet digestion is better. The values found with dry ashing are often higher. However, the differences between the results of the two methods are not significant. To ascertain whether complete recovery takes place in wet digestion, we added 8,3 $\mu$g Mn to samples nos 5 and 13 and estimated the manganese content.
The recovered values are 8,4 and 8,0 Mn respectively. From the above data we consider the wet digestion procedure preferable to the dry method.

## II. COMPARISON OF THE PERMANGANATE WITH THE FORMALDOXIM METHOD

For this study samples were digested with the acid mixture since wet digestion was found to be preferable. The permanganate method used

is described by Van Koetsveld (1958) and the formaldoxim method by Kniphorst (1946). Manganese gives with formaldoxim in alkaline environment a brown-red compound, which has according to Hofmann and Ehrhardt (1913) the formula $(CH_2NO)_3Mn.3H_2O$. Iron is removed with a zinc hydroxide suspension. However, the method has some limitations, namely excessive use of reagent interferes with the colour and a high ammonium salt concentration retards colour development which can easily be prevented by the slight precaution described in the procedure recommended. This method is sensitive to as low as 10 $\mu$g manganese. In our experience we have found that the sensitivity of the formaldoxim method is better than that of the permanganate method. The calibration factor expressed per ml of the permanganate method is 23,5 as compared with 5,2 of the formaldoxim method under the same conditions. Thus the sensitivity of the formaldoxim method is 23,5 : 5,2 = 4,5 times higher.

**Procedure recommended**

The procedure for estimation of manganese in cattle hair with the formaldoxim method is as follows.

1. *Preparation of the sample.*
   Wash the hair with warm dilute T-pol (an non-ionic detergent). Rinse with sufficient warm tap-water and finally with deionised water. Use for filtering of the hair a Buchner-funnel fitted with nylon gauze. Dry the washed hair in an oven at about 100°C.
2. *Method of estimation.*
   Reagents:
   a. *Digestion mixture:* 10 vol. $HNO_3$ (1,4), 1 vol. $H_2SO_4$ (1,84) and 1 vol. $HClO_4$ (1,67).
   b. *Zinc hydroxide suspension:* Dissolve 100 g of $ZnSO_4$ in 750 ml of water. Add 54 ml of ammonia (25%) and stir thoroughly. Filter through a sintered glass filter, having a very fine pore diameter (Leerdam L4) and wash repeatedly till free of ammonia. Transfer the residue to a beaker containing approximately 750 ml water. Stir the residue before use to obtain a pourable suspension.
   c. *Formaldoxim solution:* Dissolve 2,0 g of hydroxylaminhydrochloric acid in 40 ml water, add 1,3 ml of formalin (35%), swirl, make up to 50 ml with water and mix. Prepare this solution immediately before use.
   d. *Standard solution:* Dissolve 406 mg of $MnSO_4.4\ H_2O$ in water with the addition of a few drops concentrated $H_2SO_4$ and make up to one litre. This solution contains per ml 100 $\mu$g of manganese. Dilute before use.

*Digestion of hair*

Weigh 1-2 g of hair in a 250 ml flat bottom, narrow-necked flask and add 20 ml of the digestion mixture. When the hair is sufficiently moist, digest on an electrically heated sand-bath. After evaporation of nitric acid indicated by a brownish appearance of the residue, add once or twice a few drops of nitric acid, till the digest is clear. Allow digestion to proceed

till a small amount of liquid is present at the bottom of the flask. Cool to room temperature.

*Development of colour*

Add 10 ml of water to the cooled residue and boil on a Bunsen burner swirling continuously.
Neutralise, when the solution is at room temperature, with 25% $NH_4OH$ in the beginning and 10% $NH_4OH$ at the end using small pieces of litmus and congo-red papers as indicators. At first congo-red paper shows a change from blue to red with the addition of 25% $NH_4OH$ and with further addition of a few drops of 10% $NH_4OH$, litmus paper changes from red to blue. At this point one or two drops of 10% acetic acid are added just to bring back the colour of litmus paper to red or reddish-blue. Then 5 ml of the zinc hydroxide suspension is added to the solution, to prevent interference of iron. Filter the solution through MN 680 m filter 9 cm, into a 50 ml volumetric flask. Rinse the flask and the residue on the filter several times with deionised water. To the filtered solution in the 50 ml flask add 1 ml formaldoxim reagent, mix, and finally add 25-30 drops of $NH_4OH$ for full development of the colour. Wait at least 30 minutes before measuring the absorbance in a spectrophotometer (Beckman model or other type).

*Measurement of absorbance*

Measure the absorbance in a glass cell with a path-length of 10 mm at a wave-length of 450 nm and calculate Mn from the absorbance reading comparing with a calibration curve prepared with 10 to 200 $\mu$g Mn solutions in a volume of 50 ml.

*Precautions:*

a. Neutralisation should be carried out as precisely as possible because of the amphoteric character of the zinc suspension.
b. Blank and standard should be included in each series.
c. Both with the blank and standard, $NH_4OH$ should be added first, followed by formaldoxim reagent.

IIa. COMPARISON OF RESULTS OF THE TWO METHODS

For comparative purposes the manganese content of 35 hair samples has been estimated by both methods. The results are shown in table 3. With the formula $s = \sqrt{\frac{\Sigma d^2}{2n}}$, where d is the difference between the replicates and n is the number of samples, the standard deviation for a single analysis of both methods has been calculated. The standard deviation of the permanganate method is 1,26 (coefficient of variation = 8,3%) and that of the formaldoxim method 1,06 (coefficient of variation = 7,6%). The reproducibility of the estimated values for both methods was found to be satisfactory. Generally the permanganate method gives higher values compared with the other one. The difference, however, is not significant. From the results obtained we prefer to adopt the formaldoxim method because of its superior sensitivity and the easiness of colour development as compared with the permanganate method.

## IIb. ANALYTICAL ERROR

Extensive analytical data on manganese (773 hair samples in duplicate) enabled us to calculate the standard deviations of an individual estimate at varying levels of manganese content in the hair samples examined. This is presented in table 4.

*Tabel 3.*

*Comparison of the permanganate and the formaldoxim method (wet digestion).*

| hair sample | | p.p.m. Mn | | | |
|---|---|---|---|---|---|
| | | permanganate method | | formaldoxim method | |
| nr. | colour | $x_1$ | $x_2$ | $x_1$ | $x_2$ |
| 1 | black | 7,7 | 4,8 | 7,4 | 7,3 |
| 2 | „ | 14,5 | 14,1 | 13,5 | 14,2 |
| 3 | „ | 10,0 | 10,0 | 8,8 | 8,3 |
| 4 | „ | 11,9 | 11,9 | 10,4 | 10,5 |
| 5 | „ | 6,3 | 6,3 | 6,5 | 6,5 |
| 6 | „ | 8,2 | 8,2 | 8,8 | 8,8 |
| 7 | „ | 18,5 | 17,9 | 17,6 | 17,9 |
| 8 | „ | 10,7 | 10,7 | 9,3 | 11,1 |
| 9 | „ | 7,1 | 7,1 | 6,2 | 6,0 |
| 11 | „ | 6.0 | 6,0 | 5,4 | 6,0 |
| 12 | white | 7,7 | 4,8 | 4,9 | 6,2 |
| 13 | „ | 2,2 | 2,2 | 3,4 | 3,5 |
| 14 | red | 24,9 | 23,1 | 27,5 | 25,9 |
| 15 | „ | 23,8 | 25,6 | 24,1 | 21,8 |
| 16 | „ | 14,9 | 14,9 | 13,2 | 10,6 |
| 17 | „ | 26,2 | 24,4 | 24,1 | 24,1 |
| 18 | „ | 23,4 | 24,9 | 19,7 | 20,7 |
| 19 | „ | 28,0 | 30,4 | 24,6 | 27,2 |
| 20 | „ | 14,9 | 13,4 | 11,9 | 12,2 |
| 21 | „ | 30,1 | 29,8 | 25,7 | 28,5 |
| 22 | „ | 12,5 | 11,9 | 11,7 | 11,7 |
| 23 | „ | 24,4 | 22,6 | 20,6 | 22,5 |
| 24 | „ | 35,7 | 35,7 | 35,1 | 31,2 |
| 25 | „ | 19,1 | 20,8 | 18,3 | 18,9 |
| 27 | „ | 22,6 | 20,8 | 17,6 | 17,9 |
| 28 | „ | 6,0 | 6,0 | 4,5 | 4,9 |
| 29 | „ | 10,1 | 14,9 | 7,9 | 7,6 |
| 32 | „ | 3,6 | 4,8 | 1,8 | 1,8 |
| 33 | „ | 10,1 | 10,1 | 7,1 | 6,8 |
| 34 | „ | 5,4 | 8,3 | 10,5 | 7,9 |
| 35 | „ | 7,1 | 7,1 | 8,4 | 9,2 |
| 36 | „ | 20,8 | 22,6 | 17,2 | 18,0 |
| 37 | „ | 16,7 | 11,9 | 11,6 | 9,2 |
| 38 | „ | 11,3 | 13,1 | 11,3 | 14,2 |
| 39 | „ | 29,8 | 28,6 | 27,6 | 27,9 |

It appears from table 4 that the analytical error decreases with increasing manganese content of the sample, as is expected. The standard deviations mentioned in table 4 refer to the manganese determination made in single instead of duplicate. The laboratory normally gives the mean of duplicates. With such a practise the standard deviation will be eventually $\frac{1}{\sqrt{2}}$ times higher.

*Table 4.*

*Standard deviation (s) and in % of the mean (s%) for a single manganese determination in hair samples according to the formaldoxim method.*

| manganese content of the hair sample p.p.m. | number of samples | mean manganese content p.p.m. | S | S % |
|---|---|---|---|---|
| < 3 | 136 | 2,08 | 0,211 | 10,13 |
| 3 — 5 | 189 | 3,90 | 0,398 | 10,20 |
| 5 — 10 | 215 | 6,95 | 0,378 | 5,44 |
| 10 — 15 | 65 | 12,27 | 0,724 | 5,90 |
| 15 — 20 | 33 | 17,77 | 0,628 | 3,53 |
| 20 — 30 | 73 | 24,85 | 1,299 | 5,23 |
| 30 — 40 | 28 | 33,89 | 2,111 | 6,23 |
| ≧ 40 | 34 | 63,88 | 1,860 | 2,91 |

## III. CLEANING OF HAIR

A thorough brushing of the animal is prerequisite for good sampling. In spite of this precaution it is difficult to remove pieces of faeces and dandruff. Thus it is necessary to clean the sample in the laboratory. The common way of cleaning is washing with T-pol followed by tap water (3 times) and with deionised water (3 times). After these cleaning operations the samples are dried at 105°C. We compared the above washing procedure with washing with organic solvents like acetone or carbon tetrachloride and our results confirmed the superiority of T-pol.

The question may arise whether manganese contamination can be removed by washing. To study this point some cleaned and dried samples of hair immersed over-night in a solution of manganese sulphate in the concentration range 150$\mu$g ,300 $\mu$g and 500 $\mu$g Mn per ml. The samples were then taken out and dried at 105°C. After drying the samples were washed in two different ways:

a. The dry hair was washed with warm T-pol, rinsed repeatedly with warm tap water and finally a few times with deionised water. Finally the sample was again dried at 105°C.
b. The dry hair was washed with a warm solution of 0,5 N HCl, rinsed repeatedly with warm tap water and then a few times with deionised water. The sample was dried at 105°C.

Table 5 gives the results of the manganese estimations. It appears that manganese contamination cannot be removed by these methods.

*Table 5.*

*Influence of washings with T-pol and 0,5 N HCl on the removal of manganese contamination in the samples.*

| sample nr. | manganese content p.p.m. washed with T-pol contaminated with a solution of manganese concentration | | | | washed with 0.5 N HCl contaminated with a solution of mangenese concentration | |
|---|---|---|---|---|---|---|
| | 0 μg | 150 μg/ml | 300 μg/ml | 500 μg/ml | 0/μg | 500 μg/ml |
| E16 white | 2,5 | 211 | 217 | 390 | 0,8 | 183 |
| E37 red | 14,1 | — | — | 761 | 9,6 | 897 |
| E43 red | 11,8 | 499 | 740 | 785 | 12,5 | 537 |
| E46 white | 1,7 | 60 | 91 | 152 | 1,3 | 238 |

Table 6 gives the manganese content of the contaminated hair samples washed with a 2½% solution of ammonia. The samples were washed twice with dilute ammonia; rinsed repeatedly with tap water and finally a few times with deionised water. As shown in the table 6 this method of washing also does not ensure removal of the manganese contamination. The importance of these results will be understood in applying the method of hair analysis for diagnostic purposes. Changes of slight contamination in the sample may lead to erroneous interpretation.

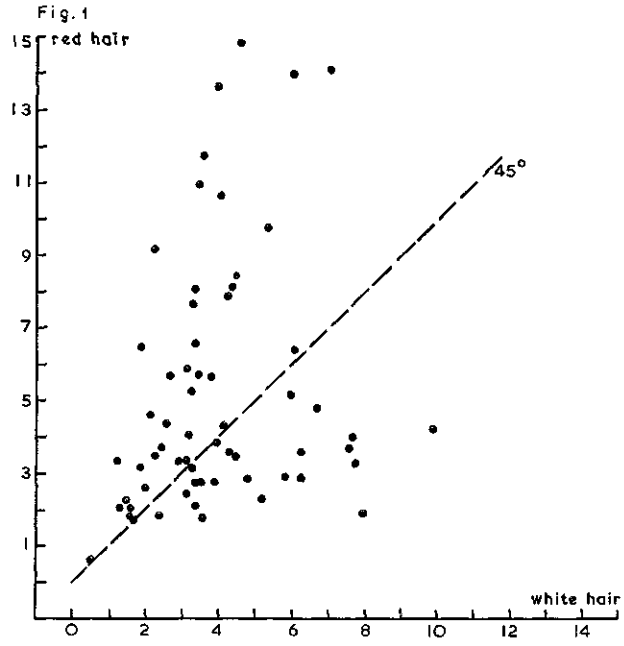
*Table 6.*

*Effect of washing with 2½% ammonia on the removal of manganese contamination introduced in the samples.*

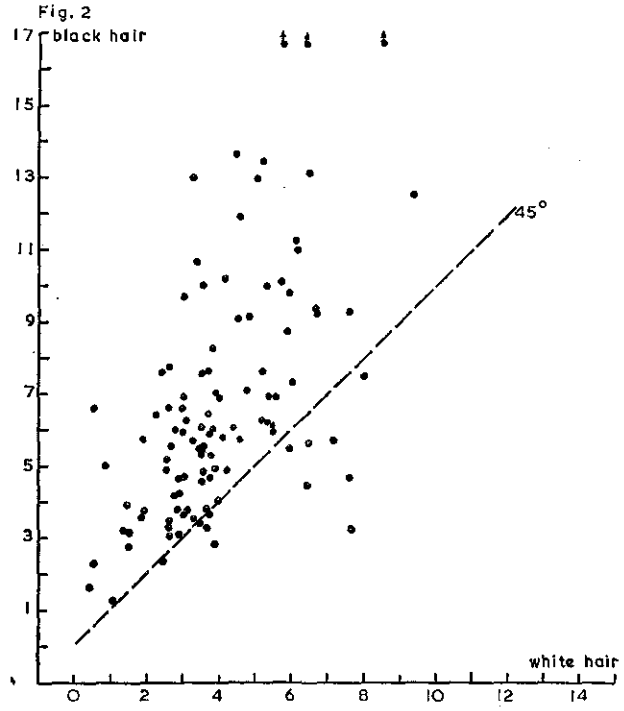
| sample nr. | colour | manganese content p.p.m. contaminated with a solution of manganese concentration | | | |
|---|---|---|---|---|---|
| | | 0 μg | 150 μg/ml | 300 μg/ml | 500 μg/ml |
| E 7 | black | 8,9 | 613 | 755 | 988 |
| E29 | red | 8,4 | — | 809 | 949 |
| E30 | black | 9,1 | — | 934 | 977 |
| E33 | red | 7,8 | 447 | 704 | 790 |

## IV. MANGANESE CONTENT OF COLOURED AND WHITE HAIRS.

Several investigators (see Van Koetsveld 1958) mention that coloured hairs contain more manganese than white ones. From 61 animals we collected paired samples of red and white hair (from the same animal both red and white hairs). Figure 1 shows the correlation between the manganese contents of both samples. For 21 animals the manganese content of white hairs is higher than that of red hairs. In all cases where the manganese content of white hairs is higher than that of red hairs, it is interesting to find that the samples are from calves (4 months old) not dieted with grass at the time of sampling. However, it was found




*Relation manganese content of white and red hair of the same animal.*




*Relation manganese content of white and black hair of the same animal.*

that the manganese content of red hairs also could be higher than that of white hairs in the case of calves not dieted with grass.
We also sampled 106 animals for both black and white hairs. The correlation between the manganese contents of both samples is shown in figure 2. It appears that only in 9 cases the manganese content of white hair is higher that that of black. In general it may be said that the manganese content of coloured hair is higher that that of white hair.
Additional study as to the cause of white hair having a higher manganese content than coloured hair, may provide us with interesting information.


## SUMMARY

In the present investigation a comparison has been made between wet digestion with a mixture of $HNO_3$, $H_2SO_4$ and $HClO_4$ in a ratio 10 : 1 : 1 and the dry ashing procedure for 48 hours. For the determination of the manganese content the permanganate method has been used. It appeared that the standard deviation of a single determination with dry ashing is higher (s % = 26,99) than with wet digestion (s % = 6,67). We consider the wet digestion preferable to the dry ashing method.
Another point of investigation was the comparison between the permanganate and the formaldoxim method using the wet digestion procedure. Though the reproducibility of estimated values for both methods has been found to be satisfactory, we prefer the formaldoxim method because of its superior sensitivity and the easiness of colour development. Extensive analytical data (773 hair samples in duplicate) enabled us to calculate the standard deviation introduced at varying levels of manganese in the hair samples examined (table 4).
In general it can be said that the manganese content of coloured hair is higher than that of white hair. In some cases, however, the reverse is true. Continued study as to the cause of the white hair having a higher manganese content than coloured hair may provide us with interesting information.
Finally we compared the washing procedure with T-pol (non-ionic detergent) and organic solvents. The results demonstrated the superiority of T-pol. It appeared however that manganese contamination is neither removed by T-pol nor by 0,5 N HCl or by 2½% ammonia. Thus chances of slight contamination in the sample may lead to erroneous interpretation.



## SAMENVATTING.

Mangaan in het haar van vee is als permanganaat-ion bepaald en tevens met formaldoxime als reagens. In beide gevallen is het materiaal zowel droog als nat gedestrueerd. In het laatste geval is een destructiemengsel benut van $HNO_3$ (1,4), $H_2SO_4$ (1,84) en $HClO_4$ (1,67) in een volumeverhouding van 10 : 1 : 1.
Bij de vergelijking van de droge en de natte destructie is het mangaan als permanganaat-ion bepaald. De natte methode bleek de voorkeur te verdienen. De standaardafwijking voor de enkele bepaling uitgedrukt in procenten van de gemiddelde waarde van 18 in duplo onderzochte monsters bedraagt nl. 26,99 en 6,67 resp. voor de droge en de natte methode.
Vervolgens is de permanganaat- met de formaldoxime-methode vergeleken. Hoewel de reproduceerbaaheid van beide bevredigend is, verdient de formaldoxime-methode de voorkeur. De gevoeligheid van de laatste is 4,5 x zo groot en moeilijkheden bij de ontwikkeling van de kleur komen hier praktisch niet voor. De mangaanbepaling is in het vervolg dan ook steeds met formaldoxime als reagens uitgevoerd.
In totaal zijn 773 haarmonsters in duplo onderzocht. Zij zijn in series met opklimmende gehalten verdeeld. Hiervan is per serie de standaardafwijking voor de enkele bepaling berekend en tevens uitgedrukt in procenten (s %) van het seriegemiddelde (tabel 4).

Het is gebleken dat het mangaangehalte van gekleurd haar hoger is dan van wit haar, hoewel ook het tegendeel is waargenomen. In dit laatste geval kan voortgezet onderzoek naar de oorzaak wellicht interessante inlichtingen verschaffen.
Ook is aandacht besteed aan het wassen van het haar; behalve T-pol zijn hiervoor organische oplosmiddelen beproefd. Het wassen met T-pol bleek het meest geschikt te zijn.
Besmetting van het haar met mangaan is niet door wassen met T-pol, noch door spoelen met 0,5 N HCl of met 2½% ammoniak te verwijderen.
Onderzoek van haarmonsters die niet onder voldoende voorzorgen genomen zijn, kan tot verkeerde conclusies leiden.


## RÉSUMÉ.

Le manganèse dans le pelage du bétail a été déterminé comme ion-permanganique et aussi avec de la formaldoxime comme réactif. Dans les deux cas le matériel a été détruit aussi bien à l'aide de la méthode sèche que de la méthode mouillée. Au dernier cas un mélange à destruction a été utilisé de $HNO_3$ (1,4), $H_2SO_4$ (1,84) et de $HClO_4$ (1,67) dans une proportion de volume de 10 : 1 : 1.
Pendant la comparaison des méthodes sèche et mouillée le manganèse a été déterminé comme ion-permanganique. La méthode mouillée se trouvait être préférable.
La déviation-standard pour la détermination simple exprimée en pourcentages de la valeur moyenne de 18 échantillons examinés en double s'élève notamment à 26,99 pour la méthode sèche et à 6,67 pour la méthode mouillée.
Ensuite la méthode permanganique et la méthode avec de la formaldoxime ont été comparéese. Bienque la susceptibilité de reproduction des deux soit satisfaisante, la méthode à la formaldoxime est préférable. La sensibilité de la dernière est de 4,5 fois plus grande et le développement de la couleur ne présente pratiquement pas de difficultés avec cette méthode. Par la suite la détermination du manganèse a donc toujours été faite avec la formaldoxime comme réactif.
En total 773 échantillons de poil ont été examinés en double. Ils ont été divisés en séries avec des teneurs ascendantes. Ensuite on a calculé la déviation-standard par série pour la détermination simple et aussi exprimé la déviation-standard en pourcentages (s%) de la moyenne par série (tableau 4).
Il a paru que la teneur en manganèse de poil coloré est plus élevé que celle de poil blanc, bienqu'on ait aussi observé le contraire. Dans ce dernier cas des recherches poussées plus loin vers la cause pourront fournir peut-être des informations intéressantes.
On a également étudié le lavage du pelage; en plus de T-pol on a éprouvé des solvants organiques à ce but. Le lavage au T-pol parut être le plus approprié.
Une souillure des poils de manganèse ne peut pas être éliminée par un lavage au T-pol, ni par un rinçage avec 0,5 N HCl ou avec 2½ % d'ammoniaque.
Un examen d'échantillons de palage, lesquels n'ont pas été prélevés avec suffisamment de précautions, peut mener à des conclusions erronées.



## ZUSAMMENFASSUNG.

Mangan im Haar von Vieh wurde spektrophotometrisch als Permanganation bestimmt und ebenfalls mittels Formaldoxim als Reagenz. In beiden Fällen wurde für das Material neben der trockenen Verbrennung das nasse Verfahren benutzt, im letzten Fall mit einer Mischung von $HNO_3$ (1,4), $H_2SO_4$ (1,84) und $HClO_4$ (1,67) in einem volumetrischen Verhältnis von 10 : 1 : 1.
Die nasse Methode hat sich als die beste bewährt erwiesen. Die Standardabweichung beträgt bei der trockenen Verbrennung nämlich 26,99% und beim nassen Verfahren 6,67% des Mittelwertes der 18 untersuchten Haarproben. Das Mangan wurde hier als Permanganation bestimmt.

Die Bestimmung des Mangans mit Formaldoxim ist dem Permanganat-Verfahren vorzuziehen, weil die Empfindlichkeit mit Formaldoxim die der anderen Methode um das 4,5-fache übertrifft. Ausserdem gibt es mit diesem Reagenz bei der Farbe-Entwicklung keine Schwierigkeiten. Es sind im ganzen 773 Haarproben mit wechselndem Gehalt mittels Formaldoxim auf Mangangehalt geprüft worden. Eine Berechung der Standardabweichung für Haar mit wechselndem Mangangehalt wurde durchgeführt (Tabelle 4).
Farbiges Haar enthält mehr Mangan als farbloses. Jedoch zeigte sich in einigen Fällen auch das Gegenteil. Weiterführung der Untersuchungen nach der Ursache dieser Ausnahmen kann vielleicht zu interessanten Ergebnisse führen.
Die Reinigung der Haarproben mit T-pol hat sich als die beste erwiesen und ist der mit organischen Lösungsmitteln vorzuziehen.
Kontamination mit Mangan ist entweder mit T-pol, verdünnter Salzsäure oder verdünnter Ammonia nicht zu entfernen. Deswegen können mit haarfremden Mangan angereicherte Materiale, also Haare die nicht unter den strengsten Bedingungen dem Tiere entnommen wurden, zu Fehlschlüsse führen.


## RESUMEN.

Se ha determinado mangano en el pelo de ganado como iono de permaganato y también con formaldoxima como reagente en los dos casos se ha destruido el material así áridamente como majadamente. En el último caso se ha usado una mixtura de destrucción de $HNO_3$ (1,4), $H_2SO_4$ (1,84) y $HClO_4$ (1,67) en une relación de volumen de 10 : 1 : 1.
En la comparación de la destrucción arida y mojada se ha determinado el mangano como iono de permanganato. Resultaba que el método mojado era preferible.
La deviación estandardo para la determinación sola, expresa en porcientos del valor pormedio de 18 muestras examinadas en duplo es a saber 26,99 y 6,67 respectivamente para el método árido y mojado.
Luego se ha comparado el método de permanganato con el método de formaldoxima. Aunque la reproducibilidad de los dos da satisfacción, es preferible el método de formaldoxima, la sensibilidad del último es 4,5 veces mayor y dificuldades en el desarraollo del color prácticamente no hay aquí. La determinación de mangano se ha hecho luego por eso siempre con formaldoxima como reagente.
Totalmente se han esaminado 773 muestras de pelo; que se han diviso en serias con contenidos subientes de esto se ha calculado por scria la deviación estandardo para la determinación sola y tambien expresa en porcientos (s%) del pormedio de la seria (tabula 4).
Ha resultado que el contenido de mangano de pelo tinto es mayor que de pelo blanco, aunque se ha observado lo contrario tambien. En este último caso más examen hacía la causa a caso puede dar informaciones interesantes.
Se ha prestado atención también al lavar del pelo; además de T-pol se han tentado para esto medios de solución orgánicos. Resultaba que el lavar con T-pol era lo más apropiado.
No se puede quitarse de una contaminación del pelo con mangano por lavar con T-pol, ní con 0,5 N HCL nî con 2½% $NH_3$.
Examen de muestras de pelo nose han tomado con cautelas suficientes, puede dar conclusiones falsas.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