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# Essential trace elements for plants, animals and humans

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NFJ Seminar no. 370
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Nordic Association of Agricultural Scientists

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# Preface

The soil is the primary source of trace elements for plants, animals and humans. Agricultural fields have been fertilized with macronutrients for decades but fertilization with trace elements has been limited. Has the trace element content of crops decreased as a result of increasing yield, depletion in soil and limited addition in inorganic fertilizers? Can yield be increased or the quality of crops improved by the addition of relevant trace elements? Fodder and food are often enriched with trace elements in inorganic form, but are inorganic forms of trace elements of the same value as organic forms for animals and humans? What trace element content is needed in crops to meet the consumer's needs?

The seminar will address these questions. The participants in the seminar should keep these questions in mind during the seminar and ask questions, give comments and participate in discussions so we can clarify where in the food chain, from soil to table, possible improvements of trace element content and quality could be beneficial.

Gudni Thorvaldsson

# Programme

## Monday August 15th

08:45 Registration and collection of proceedings.
09:15 Opening of the seminar. *Sveinn Aðalsteinsson, NJF´s President*

**Soil/plants – Chairman: Holger Kirchmann**


09:30 Trace elements in soil: status and management.
*Johnny Johnston, England* (**keynote speaker**) …………………...………… 07
10:30 Coffee break
10:50 Essential trace elements and food quality. *Lars Johnsson, Sweden* …………..... 15
11:20 Status of micronutrient demand of Danish crops. *Leif Knudsen, Denmark* ……. 18
11:50 Discussion
12:00 Lunch


**Soil/plants – Chairman: Lennart Mattsson**


13:00 Effects of soil characteristics and fertilizer application on grass yield and chemical composition including mineral and trace element content – Farm scale studies in Finland 1995-2004. *Mikko Korhonen, Finland* ………………..… 21
13:30 Nitrogen fixation by red clover as related to the supply of cobalt and molybdenum from some Norwegian soils. *Olav M. Synnes, Norway*……..…… 24
14:00 A survey of manganese deficiency in Danish agriculture.
*Leif Knudsen, Denmark* …………………………………………………….….. 25
14:30 Coffee break
14:50 Trace elements in long term experiments in Iceland and Sweden.
*Holger Kirchmann, Sweden* …………………………………………….…… 30
15:20 Discussion.
15:40 Presentation of posters.


## Tuesday August 16th

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09:00 Trace elements in animal nutrition.
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10:30 Selenium in hay and the blood of sheep in Iceland.
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11:00 Variation in hepatic copper accumulation in sheep – seasonal and genetic effects. *Tore Sivertsen, Norway* …………………………...……..….. 40
11:30 Trace element status of soil and organically grown herbage in relation to animal requirements. *Espen Govasmark, Norway* ………………………………43
12:00 Lunch
13:00 Study tour to Nesjavellir and Þingvellir.
19:30 Dinner at Hotel Loftleiðir.

## Wednesday August 17th

**Food – Chairman:**

09:00 Essential trace elements in human nutrition.
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10:00 Coffee break
10:30 Selenium supplemented fertilization – effects on the selenium content of foods and selenium intake in Finland. *Merja Eurola, Finland* ….…………………..... 49
11:00 The role of manganese application on quality of potato tubers.
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11:30 Trace element content of rye (*Secale cereale* L.) cultivars in official variety trials in Finland during 1998-2002. *Merja Eurola, Finland* …………………… 55
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**Animals/fertilizers/analysis – Chairman:**

13:00 Contents of some essential trace elements in grass from cultivated meadows and vegetation from natural pastures in Western Norway. Is there an adequate supply to sheep? *Astrid Sandvik, Norway* ……………………………….....…… 57
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14:30 Coffee break.
14:50 High-throughput profiling of micronutrients and trace elements in crops for improvement of nutritional content and traceability.
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15:20 Soil analysis – key to nutrient management planning.
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15:50 General discussion.

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# Trace elements in soil: status and management

A. E. Johnston
*Rothamsted Research*

## Introduction

There is an increasing awareness of the need to pay greater attention to the role of trace elements in plant and animal nutrition as we seek to explain the adverse effects of deficiencies and toxicities, and avoid suboptimal concentrations that limit the attainment of optimum economic yields of crops and animal productivity and welfare. The term trace element is useful but imprecise because it can refer to any element in the soil-plant-animal system regardless of its role. Originally the term was applied to the many elements found in plants at concentrations that were so small that the then available methods of analysis could only report them as "a trace", and often before any role could be assigned to them. Now with modern analytical techniques, concentrations as little as ng $g^{-1}$ can be determined but there is still uncertainty about the optimum concentration of many trace elements in plant tissue and even less information on adequate levels of plant available trace elements in soil.

Some trace elements are essential nutrients for plant growth and often also for food and feed quality because the primary route for their intake by humans and animals is plants. These trace elements might better be called micronutrients. Included in this group are boron, chlorine, copper, iron, manganese, molybdenum and zinc. The importance of other trace elements found in plants but, as yet without any recognised function, relates to their role in animal nutrition. Their presence in plants would appear to have allowed animals to use them in their metabolic processes, often in enzyme systems. Among such elements are cobalt, chromium, fluorine, iodine, nickel and selenium. Animals having developed a dependency on these trace elements they too could be described as micronutrients. More elements have been shown to be essential for animals than for plants. Thus it is essential that micronutrients, whether required by plants or animals, are present in sufficient plant-available concentrations in the soil to ensure optimum productivity.

A problem with the term trace element is that it might suggest that, even if an element is not essential for plants or animals, it has no adverse effect on either. But some elements can accumulate even as traces to concentrations that are toxic to the plant or to the animal feeding on it. For example, plant growth is adversely affected by excess manganese (Mn) and aluminium (Al) in many soils if they are allowed to become acid, or by nickel (Ni), cobalt (Co) or chromium (Cr) in acid soils derived from minerals in which there is a natural abundance of these elements. In other soils, concentrations of trace elements have increased as a consequence of human activity and such pollution often results in increased plant uptake with adverse effects on humans, as for example with cadmium (Cd). In these cases it is essential to ensure that the concentration of the element in the soil solution does not exceed an agreed critical value.

The microelements that are required in trace amounts by plants and animals and the range of concentrations of each element found in soil are in Table 1. For a general review see Adriano (1986) and Mertz (1981).

Table 1. Essential micronutrients for plants and animals. (Adapted from Wild, 1988)

| Element | Plants | Animals | Typical range of soil content[1] |
|---|---|---|---|
| Boron | Y | * | 0.9 - 1000 |
| Chlorine | Y | Y | 18 - 806 |
| Cobalt | * | Y | 0.3 - 200 |
| Chromium | * | Y | 0.9 - 1500 |
| Copper | Y | Y | 2.5 - 60 |
| Fluorine | * | Y | 6 - 7070 |
| Iodine | * | Y | <0.09 - 80 |
| Iron | Y | Y | 0.01 - 21% |
| Manganese | Y | Y | <1 - 18300 |
| Molybdenum | Y | Y | 0.07 - 5 |
| Nickel | * | Y | 0.1 - 1523 |
| Silicon (as dioxide) | * | Y | 50 - 70% |
| Selenium | * | Y | 0.03 - 2 |
| Zinc | Y | Y | 1.5 - 2000 |

Y, essential; * essentiality not yet established
[1] Units of measurement mg $kg^{-1}$ except where % is used

**Sources of trace elements in soils**

The trace element content of a soil depends initially on the parent material from which it was formed (Mason and Moore, 1982) but subsequent leaching and nutrient cycling through plants and animal excreta creates both depletion and enrichment often in specific soil horizons. The soil profile can also gain elements through deposited dust, important in areas prone to dust storms, by adsorption from water draining into a soil from elsewhere and by pollution due to human activity.

Depending on their valence and ionic radius, trace elements can become incorporated in silicate crystals and one ion can substitute for another. For substitution to occur, the ionic radius of the two ions must not differ by more than 15%. For example, the ionic radius of magnesium (Mg) is 66 pm (pm = $10^{-12}$ m) and it can be replaced by cobalt (II) (72 pm), chromium (III) (63 pm), nickel (69pm) and zinc (74pm). Consequently, soils derived from basic rocks are usually not deficient in micronutrients and crop deficiencies occur rarely. Indeed, toxicities may sometimes be a problem on such soils, for example soils derived from serpentine can contain large amounts of plant available chromium (Cr) and nickel (Ni), and on these soils a flora tolerant of the large concentrations of these two elements can be found. In contrast, acid igneous soils are generally poor in some micronutrients so that deficiency adversely affects plants and animals. For example, Co deficiencies are common on soils derived from granite or rhyolite. There is a complicating factor in relation to the forms of metallic micronutrients. If sulphur (S) is present then metal ions are preferentially bound to S atoms rather than oxygen (O) atoms. Such sulphide ore minerals when they occur in rocks near the surface of the Earth, weather and release the metals, especially copper (Cu), lead (Pb) and zinc (Zn), and they then accumulate in soil. As with the presence of large amounts of Cr and Ni, this has lead to the development of specialised, metal-tolerant flora. The ability to develop tolerance and accumulate such metals, has led to a search for plants that will remove metals from metal-contaminated soils.

Agriculturally, the main sources of trace elements are the sedimentary rocks that cover about 75 percent of the earth's surface. As the primary minerals weather, the alkaline earth elements and alkali metals tend to remain in solution and some of the metallic micronutrients pass into the lattices of the secondary or clay minerals, while others are adsorbed onto claysized particles or become incorporated into humified organic matter. Where the composition of a soil is closely similar to that of the parent material i.e. for young soils, it is possible to make some generalisations about their trace element content. For example, soils derived from shales often have a satisfactory trace element composition.

In contrast to the very broad generalisations that can be made about the likely trace element content of young soils, little general guidance can be given about the trace element composition of old soils rich in Fe, Mn and Al oxides. This is because old soils have undergone very variable degrees of weathering.

Trace elements are released into the soil solution as weathering decomposes silicate minerals and a number of factors then come into play. Trace elements may precipitate immediately or remain in solution depending on the ratio of ion charge to radius (in nm), often called the ionic potential (IP). Elements with ratios above 95 form oxyanions and they include B, Cr (VI), Mo (VI) and silicon (Si). Elements like Cd, Co, Cu, Mn (II), Ni and Zn have IP values below 30 and form soluble cations. These soluble cations may become "trapped" in precipitates of compounds derived from elements with IP values between 30 and 95. Elements in this group include Cr (III), Fe (III), Mn (III), Mn (IV) and Mo (IV). They tend to accumulate as precipitated hydrous oxides in the residues from weathered silicate minerals and are found as discrete particles or as a coating on soil crumbs and the soil around voids within soil. The chemical control that these hydrous oxides exert on the action of other ions far exceeds what might be expected from their concentrations (Jenne, 1986). As the hydrous oxides are formed, other trace elements can be occluded in the precipitate (Taylor and Giles, 1970). For example, both Ni and Co are associated with Mn oxides and Cu and Zn with both Mn and Fe oxides. As pH increases, the hydrous oxides have strong adsorption affinities at their surfaces for trace cations and after adsorption the ions can migrate by solid state diffusion into the clay lattice on which the oxide is held. Hydrous oxides can also dissolve and then precipitate again in response to changes in soil pH and redox potential and this can cause changes in the plant availability of the trace elements. The plant availability of many micronutrients depends on soil pH. Boron, Cu and Zn are plant available over a soil pH range of 5 to 7 but Fe and Mn have greater availability below pH 6 whilst Ca and Mg are more available above pH 6.5.

Under undisturbed vegetation, especially on light textured soils, the distribution of trace elements in the soil profile is strongly influenced by their uptake by plants from a large volume of soil and their subsequent return to the surface in leaf fall. Under these conditions, trace elements are retained initially in association with organic matter (Swain and Mitchell, 1960), but as the humus decomposes the trace elements can move down through the soil profile in drainage water and be lost or they can be retained in clay rich horizons lower down the profile. When such light textured soils are reclaimed for agricultural use micronutrient deficiencies can occur because soil cultivation increases the rate at which humus decomposes and the biogeochemical cycling of the elements is disrupted when they are removed in the harvested produce.

**Availability of micronutrients to plants**

In general, the total content of a micronutrient in soil is only a very broad guide to its plant availability. In consequence, many chemical solutions have been tested as extractants for removing from soil amounts of micronutrients that relate to their availability to plants, and from the data indices of their plant availability have been developed. The data can be used in three ways. (i) To identify soils where a response to an application of the element in question might be expected or to identify or confirm a micronutrient deficiency following an observation that lack of an element might be the cause of poor growth. (ii) To identify soils, especially contaminated soils, where crop uptake of an element could lead to problems of food/feed quality, e.g. soils with too large concentrations of Cd, Pb or arsenic (As). (iii) To identify the major soil pools of micronutrients (and toxic metals) by a chemical fractionation of the soil.

The same extractants can be used for all three purposes. Although no extractant is based on any firm theoretical foundation, all have proved of practical assistance in both advisory work and laboratory studies. Some of the more commonly used reagents are listed in Table 2 in the order in which they are used for sequential extraction and this relates to their increasing ability to extract more firmly bonded micronutrients and those in less soluble forms.

Table 2. Some common soil extractants used to extract micronutrients from soil and the chemical forms they are thought to extract. (Adapted from Wild, 1998)

| Soil fraction | Common extractant |
|---|---|
| Soil solution | $H_2O$; 0.01 M $CaCl_2$ |
| Readily exchangeable | 0.5 M $CH_3COONH_4$; 0.2 M $MgSO_4$ |
| | 0.1 M $NH_4$ Cl; 1 M $NH_4NO_3$ |
| Specifically sorbed | 0.5 M $CH_3COOH$; 0.1 M HCl; 0.1 M $HNO_3$ |
| Organically bound | 0.05 M EDTA[1]; 0.05 M EDDHA[2]; |
| | 0.005 M DTPA + 0.1 M TEA + 0.01 M $CaCl_2$ [3] |
| Hydrous oxide bound | 1 M $CH_3COONH_4$ + 0.002 M $C_6H_6O_2$[4] |
| | 0.2 M $(COO)_2 (NH_4)_2$ + 0.15 M $(COOH)_2$ at pH 3.3 |
| Residual | HF; Mixtures of hot concentrated acids |

[1] Ethylene diamine tetraacetic acid, disodium or diammonium salt.
[2] Ethylene diamine di-(*o*-hydroxyphenyl acetic acid).
[3] Diethylene triaminepentaacetic acid and triethanolamine with calcium chloride at pH 7.3.
[4] Hydroquinone (1:4 dihydroxybenzene).

The concentration of micronutrients in the soil solution, from which plant roots take them up, is usually at the micromolar level. But micronutrient ions rarely exist in the soil solution in simple forms; they are usually complexed with both inorganic and organic ligands. Thus there has been much work on chemical speciation of micronutrients and complex computer programmes have been written to predict the main species in very dilute solutions. Table 3 lists the principal chemical species for some elements in aerobic soils in acid and alkaline conditions and the order from left to right is approximately that of decreasing concentration.

Table 3. Principal chemical species of trace metals in acid and alkaline soil solutions in aerobic soil conditions.

| | Principal species | |
|---|---|---|
| Metal | Acid soils | Alkaline soils |
| Mn(II) | $Mn^{2+}$, $MnSO_4$, Org* | $Mn^{2+}$, $MnSO_4$, $MnCO_3$, $MnHCO_3^+$ |
| Fe(II) | $Fe^{2+}$, $FeSO_4$, $FeH_2PO_4^+$ | $FeCO_3$, $Fe^{2+}$, $FeHCO_3^+$, $FeSO_4$ |
| Ni(II) | $Ni^{2+}$, $NiSO_4$, $NiHCO_3^+$, Org | $NiCO_3$, $NiHCO_3^+$, $Ni^{2+}$ |
| Cu(II) | Org, $Cu^{2+}$ | $CuCO_3$, Org |
| Zn(II) | $Zn^{2+}$, $ZnSO_4$ | $ZnHCO_3^+$, $ZnCO_3$, $Zn^{2+}$, $ZnSO_4$ |

**Nutrient uptake and root characteristics**

As plant roots take up nutrients from the soil solution they are replenished by mass flow or by diffusion in response to the concentration gradient caused by depletion at the root surface (Davies, 1980). The processes that regulate nutrient uptake characteristics are not well understood and are specific for each ion. In general, for a given external concentration, the inflow is greater when the concentration within the plant is small than when it is large. To determine the effect of the nutrient composition of the plant on uptake characteristics requires very carefully conducted experiments (Junk, 1970) and will not be discussed further here.

*Root exudation and the rhizosphere*

The immediate vicinity of a plant root, the rhizosphere, is important for nutrient uptake although it extends only 1-2 mm from the root surface. Within the rhizosphere are numerous microorganisms whose existence depends on the release of organic and inorganic material from the root. Whether organic compounds such as acids and chelates produced by rhizosphere organisms have any major influence on nutrient availability by dissolving compounds within the soil or by chelation of some elements making others more available is still an open question. It has been postulated that elements like Fe, Cu and Zn that readily form chelates could be made more available to higher plants.

The pH of the rhizosphere will affect nutrient availability and the form in which nitrogen (N) is supplied to the plant affects rhizosphere pH. Compared to the bulk soil outside the rhizosphere, the pH of the rhizosphere can be increased by one pH unit when nitrate N is applied and it can be decreased by one pH unit when ammonium N is applied. These pH changes are important for phosphorus (P) supply and may be for micronutrient supply also. There is evidence that rhizosphere pH is decreased when plants are suffering from a shortage of Fe (Marschner, 1983), the decrease in rhizosphere pH increases its availability.

Organic acids exuded by roots have been shown to increase the availability of some micronutrients. For example exuded malate ions appear to be important in the solubilization of $MnO_2$. Jauregui and Reisenauer (1982) have suggested that $MnO_2$ is reduced by exuded malate and chelation of the $Mn^{2+}$ produced prevents its re-oxidation and increases its mobility in the rhizosphere.

Sites of localised low pH can occur in the rhizosphere. Within the root clusters (proteoid roots) of certain plants there can be an intensive extraction of nutrients from a limited soil volume because the exudates do not diffuse into a large volume of soil. Citric acid is the dominant compound in the proteoid root exudates of white lupin, *Lupinus albus*, (Gardner *et al.,* 1983) and is effective in mobilising Fe, Mn and Zn in the rhizosphere and this is an efficient way of increasing the uptake rate of these elements and their content in the plant. (Marschner, 1995).

*Mycorrhizae and the mineral nutrition of their host plant*

For micronutrients that occur in very small concentrations in the soil solution and frequently tend to be immobile, there is evidence that mycorrhizal fungi can play an important role in the uptake of some elements as judged by distinct improvements in growth often seen in plants with mycorrhizal associations. Mycorrhizal fungi occur in soils in close association with plant roots and are divided into two groups, ectotrophic and endotrophic mycorrhizae. Ectotrophic mycorrhizae (ECM) cover roots and rootlets with a thick mantle of hyphae that spread between the cortical cells of the roots ensuring close contact with the root. These fungi are mainly found on the roots of trees and shrubs and are of economic importance for the growth of forest trees.

Many plant species, including most agricultural crops, have endotrophic mycorrhizae of which vesicular arbuscular mycorrhiza (VAM) is the predominant type of fungal infection. The fungus has mycelium that actually penetrates cells in the root cortex and these are connected to an external mycelium in the root rhizosphere and soil. Both in the cortex and the rhizosphere the hyphae branch extensively and the fine hyphae can enter pores within the soil that are too small for root hairs to enter. Thus they can access nutrients that would otherwise be unavailable to the plant.

For both ecto- and endo-mycorrhiza, the symbiotic association of host and fungus is such that only living roots are infected and the infection does not damage the root. The host provides the fungus with life-sustaining organic compounds while the fungus assists the roots in exploiting the soil for water and inorganic nutrients. These beneficial effects have been extensively studied in relation to the phosphorus (P) nutrition of crops (Tinker, 1980). However, VAM appear to be of particular importance for leguminous species because their presence enhances N fixation, perhaps because the fungi increase the uptake of Co, Mo, Cu and Fe, elements known to be involved in N fixation. Besides pasture and forage legumes, most crop species, including onions, maize, wheat, barley and many vegetables, have VAM associations, the most notable exceptions are brassicas and sugar beet.

The benefits of mycorrhizal associations in the uptake of micronutrients have been studied less than those for P. The uptake of Zn and Cu are usually larger in plants with VAM than in those without (Kothari *et al.,* 1990a,b). The uptake of these two elements by the external hyphae of VAM can account for about 50-60% of their total uptake in white clover and 25% in maize; and there is evidence that the plant regulates the uptake of P and Cu separately (Kothari *et al.,* 1990a,b; Li *et al.,* 1991). As a consequence of the greater uptake of Zn and Cu by VAM plants, the shoot contents are larger than those of non-mycorrhizal plants. It has also been observed that VAM may enhance Zn toxicity in the host plant when there is a large external supply of Zn (Schuepp *et al.,* 1987).

In contrast to the benefit bestowed by VAM in increasing the uptake of Zn and Cu, the shoot content of Mn is often much less in VAM plants. In red clover there is a distinct negative correlation between percent root colonisation with VAM and the Mn content of roots and shoots (Arines *et al.,* 1989). There are two possible mechanisms for this, a lack of sufficient uptake and transport of Mn in the external hyphae and/or possible VAM-induced changes in rhizosphere microorganisms in general and a decrease in the population of Mn reducers in particular.

*Role of mycorrhizae in heavy metal tolerance*

The term heavy metal is not rigorously defined but is usually applied to those elements having a density greater than 6000 kg per cubic metre. Using this definition, the following elements, important as micronutrients or as pollutants in agriculture, could be classed as heavy metals: Cd, Cr, Co, Cu, Fe, Mn, Pb, Mo, Ni and Zn. Some clearly are plant nutrients but when present in excess, these metals and others are considered as pollutants because they adversely affect crop growth or the health of animals that feed on the plants. Research shows that many ectotrophic mycorrhizal (ECM) fungi are effective in enhancing heavy metal tolerance of the host plant (Wilkins, 1991; Colpaert and van Assche, 1993) provided that the concentration is not so large as to be directly harmful to the fungus. The mechanisms by which protection may be achieved are interesting. Preferential binding of heavy metals may occur in the mucilage on the hyphal surface, in the fungal cell walls and/or in the cell vacuoles. See Marschner (1995) for a full discussion because much of the work on ECM fungi is applicable to trees and shrubs rather than to agricultural crops.

In contrast to ECM, there are only a few reports of the effect of VAM on heavy metal tolerance of the host plant. In view of the morphological differences between ECM and VAM and the possible mechanisms involved in heavy metal tolerance by ECM, corresponding direct ameliorating effects of VAM would not be expected, although the alleviation of Mn toxicity by VAM has been referred to above. Vesicular arbuscular mycorrhizae may have indirect effects by improving growth through increased P supply, and thereby any metal taken up would be diluted through a larger mass of tissue to levels at which no adverse effects would occur.

## Concluding remarks

There is an increasing awareness of the need to assess plant micronutrient requirements and their availabilities in soil as deficiencies of macronutrients are corrected. The quantity of a micronutrient that is available for plant uptake is invariably very much less than the total amount in soil and the availability of each micronutrient depends on the form – mineral or organic complex – in which it can be taken up by plant roots. A complicating factor of great importance is the fact that the relative proportions of the forms in which micronutrients occur in soil can change with soil pH and redox potential and the amount of soil organic matter. Thus the availability of micronutrients changes with soil conditions and this makes generalisations extremely difficult. Improvements in analytical techniques for determining micronutrients in plant tissue and available forms in soil in recent years suggest that refinements in estimating critical concentrations in plants and soils should be possible. With such information, correcting deficiencies, recognising toxicities and defining suboptimal micronutrient levels in soils and plants should ensure that no nutrient limits the attainment of optimum economic yields of crops and performance of animals. But to achieve this will also require that the best forms and ways of delivering micronutrients, i.e. by foliar application or by addition to the soil, are adequately researched. This paper attempts to summarise very briefly a paper by Johnston (2004) which, in turn, was dependant on material taken from the general bibliography and the references given below.

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# Essential trace elements and food quality

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## Background

Essential trace elements or micro-nutrients such as cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo) and selenium (Se) are elements necessary for maintaining the life processes in plants and/or animals including humans. The required amounts of trace elements are much lower than the required amounts of macronutrients such as Ca, Mg, K, N and P. For most essential trace element a to low uptake/intake causes deficiency and a to high causes toxicity. The range of the interval between deficiency and toxicity differs between trace elements. In Sweden and Scandinavia the availability of trace elements is generally to low than to high.

The plant availability of trace elements in the soil, or the capability of the soil to deliver essential trace elements to agricultural crops is essential for our ability to produce high quality agricultural products. This is especially true for production systems where the farmers are mainly depending on the delivery capacity of trace elements of their own soil and of local production of organic fertilizers like farm manure. This mean that the need of knowledge about the processes regulating the availability of essential trace elements, which to date is insufficient, will increase in a near future due to a larger part of organic farming and animal production. A reduced use of mineral fertilizers, that normally contains small amounts of trace elements (Andersson, 1977a; Gissel-Nielsen, 1971), and an increased use of farm and green manure produced on the individual farms may cause a reduced addition and an increased retention and as a result reduced plant availability of essential trace elements (Andersson, 1976; Johnsson, 1991).

## Factors regulating plant availability of trace elements

The soil content of trace element in Swedish agricultural soils shows a large variation (Table 1).

Table 1. Total concentration of Cu, Co, Mn, Se and Mo in the surface layer of Swedish agricultural soils (mg $kg^{-1}$; n=3067 for Cu, Co, Mn and Mo; n=3060 for Se) (Eriksson et al., 1997)

| | Cu | Co | Mn | Se | Mo |
|---|---|---|---|---|---|
| Minimum | 1.1 | <1.0 | 18 | <0.05 | 0.06 |
| Maximum | 102.1 | 2.38 | 6209 | 13.34 | 96.68 |
| Mean value | 14.6 | 1.4 | 422 | 0.31 | 1.28 |

The plant availability of the essential micronutrients Cu, Co, Mn, Se and Mo depends on genetic differences between plant species and on soil properties such as parent material composition, organic matter content, clay content, sesquioxide content and soil pH. The amount of easily extractable Cu, Co, Se and Mo is only about 1-2 % of the total amount. For Mn the water extractable amount is somewhat higher, ca 7% (Karlsson, 1961; Andersson, 1977b; Johnsson, 1992). Accordingly, the major part of macronutrients is bound by the soil solids.

Due to the large variation in soil types between regions and between farms within the same region the plant uptake of micronutrients will vary. The differences in the cereal concentration of Co, Cu, Mo, Mn and Se presented in Table 2 can be seen as one indication on the range of the capacities of Swedish agricultural soils to deliver micronutrients to crops, but it also reflects present and past differences in nutrient management.

| Table 2. Trace element concentration in Swedish winter wheat, barley and oats (mg $kg^{-1}$) (Eriksson et al., 2000; Johnsson et al., 1997) | | | | | |
|---|---|---|---|---|---|
| Crop | Cu | Co | Mn | Mo | Se |
| Winter wheat | (n=606) | (n=606) | (n=606) | (n=606) | (n=128) |
| Range | 1.28-6.91 | <0.001-0.088 | 5.8-110.4 | 0.12-18.31 | 0.003-0.203 |
| Mean value | 3.89 | 0.0052 | 30 | 1.16 | 0.018 |
| Barley | (n=327) | (n=327) | (n=327) | (n=327) | (n=15) |
| Range | 1.35-9.71 | <0.001-0.156 | 6.5-44.2 | 0.08-12.01 | 0.005-0.084 |
| Mean value | 4.72 | 0.0051 | 16.7 | 1.11 | 0.022 |
| Oats | (n=208) | (n=208) | (n=208) | (n=208) | - |
| Range | 1.31-6.20 | 0.003-0.312 | 14.4-114.6 | 0.09-3.72 | - |
| Mean value | 3.71 | 0.017 | 46.9 | 0.87 | - |

To date the concentration of trace elements such as Cu, Co, Se and Mo in crops (Table 2) is usually also lower than the requirements/recommendations for ruminants and pigs and in purchased feed these elements are added as mineral salts (Simonsson, 1994, Viuf, 1999; Spörndly, 1999). A reduction of the trace element concentration in forage crops may increase the risk for deficiency for domestic animals. According to Jan Luthman (Dept of Ruminant Medicine and Epidemiology, SLU, personal communication) the since 1980 rare symptoms of selenium deficiency has again been reported among animals from a few ecological farms.

**Fertilization with trace elements**

If the trace element contents in forage crops and cereals are considered to low, addition via fertilizers may be a possible counter-measurement. In Finland mineral fertilizers have been supplemented with Se since 1984. A fertilization corresponding to ca 8 g per ha and year has resulted in 2-3 times higher Se intake to the Finish population compared to the intake before the start of the fertilization (Johnsson et al., 1997).

The effect of Se addition via mineral fertilizers one the Se content in cereals is clearly shown by the result from an investigation by Kivisaari (1988) (Table 3). The use of Se supplemented mineral fertilizers has increased the Se content. The content in cereals from farms where only organic fertilizers have been used was on the same level as before the supplementation started (Johnsson et al 1997) and much lower than the Se content in Swedish cereals (Table 2).

| Table 3. A comparison of the Se content and (± standard deviation) Finish cereals from farms where Se supplemented mineral fertilizers have been used and from farms where only organic fertilizers have been used (Kivisaari, 1988) | | | | |
|---|---|---|---|---|
| | Se supplemented fertilizers | | Organic fertilizers | |
| Crop | n | mg Se $kg^{-1}$ | n | mg Se $kg^{-1}$ |
| Spring wheat | 18 | 0.060 ± 0.024 | 10 | 0.010 ± 0.003 |
| Barley | 40 | 0.068 ± 0.013 | 14 | 0.008 ± 0.006 |
| Oats | 34 | 0.068 ± 0.014 | 22 | 0.007 ± 0.002 |

However, due to the large variation in soil properties soils in some areas may have the capacity to produce crops with rather high levels of trace element. For example, a few samples of pasture grass from Jämtland, Sweden, had a Se concentration of over 0.1 mg $kg^{-1}$ (Johnsson, 1992). In this area the soils are derived from shale, known to contain high levels of Se and other trace elements (Eriksson et al., 1997; 2000). Other areas in Sweden with a similar parent material include parts of Östergötland and Västergötland. In these types of areas trace element fertilization should be avoided and the additions of trace elements to feeds may be reduced or even be stopped.

**Trace element uptake by different crops**

Oats seams to contain higher concentrations of Co and Mn than winter wheat (Table 2). For Se cereals are considered to be so called non-accumulating plants that normally contain low Se levels. The highest Se concentrations are found in clover, onions and different species of cabbage. Plants that normally have high sulfur content also tend to have a higher Se content than other plants. This may be the reason way forage crops seams to contain some what higher, but sill low, Se content than cereals (Johnsson et al., 1997).

**Conclusions**

The trace element content of Swedish agricultural soils and crops show a large regional and local variation.

To date the concentrations of trace elements such as Co, Cu, Mo and Se in crops are lower than requirements /recommendations for ruminants and pigs purchased feed these elements are added as mineral salts. A larger part of locally produced feed will also increase the need of understanding about the processes regulating the delivery capacity of trace elements from different soils to our crops.

There is an increasing risk for lower trace element concentration in forage crops and cereals produced in a future agricultural system which to a greater degree than to date will be depending on the delivery capacity of the local soils and of local production of organic fertilizers.

A reduction of the trace element concentration may result in an unhealthy low intake to domestic animals and humans and an increased risk for deficiency related symptoms and diseases.

Fertilization seams to be is possible methods to achieve suitable concentration levels of essential trace elements in agricultural crops.

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# Status of micronutrient demand of Danish crops

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**Introduction**
Application of micronutrients to crops has received a great deal of attention in Denmark in recent years. The recommended application of micronutrients has not changed for many years. Besides the manganese problems, which are not described in this abstract, the recommendation has been to apply copper (Cu) if soil tests show a low copper content and to apply boron (B) to oil seed rape and other crucifers and sugar beets on sandy soils with a high pH. Deficiency in other micronutrients in agricultural crops is very uncommon. Compared to countries like Germany it is not common in Denmark to use a multi-component mix of micronutrients as a standard foliar application. Fertilizer companies have claimed that there is a demand for micronutrients in Denmark which not are covered by the existing recommendations. In 2004 investigation was carried out to determine the variation in micronutrients in soils and crops and to show whether the application of one or more micronutrients would lead to a positive yield response. The first results of this investigation showed a relatively low content of copper and zinc in many crop samples, but the analysis of the data is continuing.

**Micronutrients**
The following is a summary of each micronutrient as seen by the advisory service in Denmark.
*Copper (Cu):* Deficiency of copper in Denmark has been very widespread, especially in sandy soils with a high content of organic matter. In the 1950$^s$ and 1960$^s$ the advisory system conducted out a considerable number of trials with application of copper. On sandy soils with a low copper content the application of copper sulphate, for example, produced a very high yield (Hansen, 2003). Currently the recommendation in Denmark is based on soil samples taken every 5-7 years. Soil samples are important, especially on sandy soils without application of animal manure. Application of animal manure, especially pig slurry copper, produces a surplus of copper and therefore no need for soil samples. Testing for copper in soils is done by extracting with EDTA, and a content of less than 2 ppm copper is regarded as low.
*Zinc (Zn):* Zinc deficiency has not been observed in Denmark in agricultural crops and therefore there is no recommendation for soil sampling for zinc deficiency or to apply zinc to the crops. Most attention has been to maize, which is very susceptible to zinc deficiency. Most likely zinc deficiency will occur on the same soils where there is the greatest risk of copper deficiency. Recent soil testing and plant analyses in Denmark have shown that zinc can occur in concentrations low enough to make zinc a limiting factor in crop growth. There have been trials with foliar application of zinc in winter wheat and in maize, but no significant influence on yield has been obtained. Symptoms of zinc deficiency in maize are quite clear and from experience in the corn belt in the USA, where zinc deficiency is very common, it has been shown that before zinc application can result in a yield response the zinc-deficiency symptoms of the plants must be very clear. In Denmark maize is normally used for silage and is applied as a 30-50 ton slurry per ha. With this application of slurry the supply of zinc will normally exceed the uptake of zinc by the crop. In 2005 DAAC conducted trials with application of zinc to maize and cereals in general (Knudsen, 2005).
*Boron (B):* Deficiency of boron is common in Denmark in oil seed rape and other crucifers and in beets. In cereals the need for boron is very low and is not likely to be seen in temperate

regions. Normally the recommendation for application of boron is based on crop type, soil type and soil pH and not on testing soil samples for boron. The recommendation today is to apply boron to oil seed rape or other crucifers if the soil pH is greater than 6.5 and the soil is less than 10 % clay. To some degree application of boron can be based on experience from a single field because the symptoms of boron deficiency are quite clear. The symptoms will be more pronounced in dry years. Application of boron is normally done with a compound fertilizer or with a boron solution spray. Until now 0.02 % boron has been added to Danish-produced nitrogen fertilizer. Therefore not much attention has been given to boron (Knudsen, 2005).

*Molybdenum (Mo):* In contrast to boron, copper and zinc deficiencies, molybdenum deficiency occurs on acid soils. Crucifers, beets and especially legumes can suffer molybdenum deficiency, but the deficiency is very uncommon in Denmark in all crops and occurs only on acid soils with a high content of iron. There is no recommendation for applying molybdenum to agricultural crops, but cauliflower is normally treated with a solution of ammonium molybdate before planting (Knudsen, 2005).

*Iron (Fe):* Iron deficiency is uncommon in Denmark. The deficiency can occur in high moorland areas. The symptoms are quite clear, especially in oats. There is no general recommendation for application of iron to Danish crops (Knudsen, 2005).

**Micronutrients in organic manure**

In Denmark most of the agricultural land is fertilized with animal manure. The focus on fertilization with animal manure has been on nitrogen and phosphorus and the other macronutrients. Animal manure is, however, also an important source of micronutrients because most of the micronutrients in the feed are excreted by the animal in the manure. Besides the natural content of micronutrients in the feed, different micronutrients are added in minerals to the feed. Copper and zinc in pig production is added to ensure the animals' health and weight increase. Danish legislation sets maximum values for the amount of copper and zinc that can be added to the feed to avoid an excess of these nutrients in the slurry as compared to crop uptake. Slurry analyses (table 1) show values very similar to the calculated content of the feed and that there is still a significant excess of copper and zinc in the slurry compared to plant uptake. (Jensen, 2005; Tybirk, 2003; Poulsen, 1998).

Table 1. Application of micronutrients with slurry calculated from an application of 170 kg nitrogen per ha with slurry from cattle and 140 kg nitrogen per ha from pig slurry (Knudsen, 2005) and typical uptake in crops (cereals), g $ha^{-1}$.

| | ***Copper*** | ***Zinc*** | ***Manganese*** | ***Boron*** |
|---|---|---|---|---|
| Cattle slurry | 383 | 745 | 100 | 180 |
| Pig slurry | 586 | 1717 | 80 | 90 |
| Typical crop uptake in grain and straw (cereals) | 50 | 300 | 270 | 40 |

The field balance of micronutrients therefore varies even more than of micronutrients between those fields receiving animal manure and those fields without. On fields receiving animal manure deficiency of copper and zinc will not be likely. However, a deficiency in boron and manganese is much more dependent on the pH of the soil (and oxidation level for manganese) than the amount of the nutrient in the soil and therefore the deficiency of these nutrients is not influenced by application of organic manure to the same degree.

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# Effects of soil characteristics and fertilizer application on grass yield and chemical composition including mineral and trace element contents - farm scale studies in Finland during 1995 - 2004

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Farm scale studies on fertilizer application have been conducted during the last two decades by Finnish fertilizer company Kemira GrowHow Ltd (former Kemira Agro) in various parts of Finland. Since mineral and trace elements are important for the growth of grasses and they affect the feeding value of grasses, also mineral and trace element contents of the crops were analyzed. The aim of this study was to analyse the effects of soil characteristics (according to Finnish soil classification) and fertilizer application on grass yield, feeding value and mineral and trace element contents.

The data contains observations from farm scale studies conducted in 34 farms in 1995 – 2004. The swards were generally mixtures of timothy and meadow fescue. Data includes observations from both first- and second-cut of grass (harvest dates, fertilizer application including N, P, K and other nutrients, grass yield, digestibility, CP content, and mineral and trace element contents). The soil analysis was conducted at least at the beginning of the experimental period, which in the case of grasses usually lasted for three years in the same plot. The average N, P and K supplies were 85 (0-144) and 69 (0-130), 12.5 (0-33) and 8 (0-19.5), 25 (0-83) and 23 (0-92) kg/ha for the first and second cut, respectively. Since the fertilizers used were NPK fertilizers, increasing the N level also increased P and K supplies. The NPK fertilizers used in some cases also included other minerals and trace elements, e.g. S, Ca, Mg, Na, B, Zn and Se, so that also supply of these nutrients changed according to N level. Statistical analysis concerning the effect of N fertilizing on grass yield and quality parameters was carried using MIXED procedure of SAS. Year within the farm was used as a random factor in the model. Using this approach within farm effect can be investigated.

Some soil characteristics are shown in Table 1. Both soil pH and nutrient concentrations showed large variation reflecting the typical situation in practical farms. According to the Finnish soil classification, the mean values were around the average of the soil status scale.

Table 1. The description of the soil status in the data.

| | Mean | S.D. | Min | Max |
|---|---|---|---|---|
| pH | 6.2 | 0.44 | 5.2 | 7.1 |
| Ca, mg/l | 1536 | 652.8 | 484 | 3720 |
| K, mg/l | 137 | 65.3 | 6 | 289 |
| P, mg/l | 14 | 8.3 | 4 | 104 |
| Mg, mg/l | 235 | 120.7 | 51 | 618 |
| B, mg/l | 0.6 | 0.35 | 0.3 | 1.7 |
| Cu, mg/l | 4 | 2.3 | 0.4 | 11 |
| Mn, mg/l | 29 | 29.2 | 3 | 135 |
| Zn, mg/l | 3.9 | 3.24 | 0.7 | 26 |
| Na, mg/l | 35 | 21,8 | 8 | 89 |
| S, mg/l | 18 | 4.5 | 9 | 22 |

Table 2. Dry matter yield, D-value, CP and mineral contents of forage in both cuts.

| | n | Mean | S.D. | Min | Max |
|---|---|---|---|---|---|
| DM kg/ha | 336 | 6588 | 2739.0 | 1150 | 13749 |
| CP g/kg DM | 336 | 152 | 30.9 | 66 | 233 |
| D-value g/kg DM | 160 | 664 | 24.2 | 582 | 729 |
| P g/kg DM | 321 | 3.1 | 0.41 | 2.2 | 5.0 |
| Mg g/kg DM | 326 | 1.9 | 0.51 | 0.99 | 4.0 |
| K g/kg DM | 336 | 28.9 | 4.51 | 17.1 | 38.7 |
| Na g/kg DM | 336 | 0.2 | 0.15 | 0.03 | 1.2 |
| Cu mg/kg DM | 311 | 7.0 | 1.62 | 3.3 | 12.0 |
| Zn mg/kg DM | 310 | 31.8 | 9.32 | 17.4 | 82.6 |
| Ca mg/kg DM | 311 | 4.0 | 1.11 | 2.1 | 10.8 |
| Mn mg/kg DM | 115 | 49.4 | 21.29 | 19.3 | 150 |
| Fe mg/kg DM | 120 | 99.9 | 34.52 | 51.2 | 351.0 |

The mean dry matter yield was relatively high and showed large variation due to varying annual growth conditions and amount of fertilizers supplied (Table 2). In Finland, grass silage is harvested usually twice during the growing season. The proportion of cuts from the total grass dry matter yield varies and depends on annual weather conditions, timing of harvest and fertilizer application. The mean proportion of the first cut in this data was 52 % (range 25-85 %). The concentrations of minerals and trace elements were at the level typically found in Finland. Again the variation was high.

Table 3. The yield and concentration of N, minerals and trace elements in forage (first and second cut averaged) in respect to varying nitrogen fertilization (kg/ha).

| | Intercept | S.E. | P-value | Slope | S.E. | P-value | RSME | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| Yield, kg DM/ha | 3426 | 188.1 | *** | 20.8 | 0.84 | *** | 615.7 | 0.88 |
| N, g/kg DM | 18,3 | 0.45 | *** | 0.039 | 0.0022 | *** | 1.66 | 0.79 |
| P, g/kg DM | 2,95 | 0.046 | *** | 0.001 | 0.0002 | *** | 0.131 | 0.42 |
| K, g/kg DM | 25,2 | 0.39 | *** | 0.025 | 0.0020 | *** | 1.76 | 0.58 |
| Ca, g/kg DM | 3,8 | 0.18 | *** | 0.001 | 0.0007 | 0.11 | 0.337 | 0.07 |
| Mg, g/kg DM | 1,7 | 0.07 | *** | 0.001 | 0.0003 | *** | 0.146 | 0.32 |
| Na, g/kg DM | 0,17 | 0.013 | *** | 0.0004 | 0.00008 | *** | 0.083 | 0.14 |
| Fe, mg/kg DM | 102 | 11.3 | *** | -0.021 | 0.0579 | 0.72 | 26.9 | 0.01 |
| Cu, mg/kg DM | 6,0 | 0.23 | *** | 0.007 | 0.0011 | *** | 0.67 | 0.40 |
| Mn, mg/kg DM | 51,1 | 5.48 | *** | 0.010 | 0.0156 | 0.53 | 7.2 | 0.01 |
| Zn, mg/kg DM | 26,0 | 0.95 | *** | 0.037 | 0.0047 | *** | 4.4 | 0.31 |

The regression equations describing the responses to varying N fertilization are presented in Table 3. As expected, there was a significant positive linear relationship between N fertilizing level and grass yield and grass N content. The slope of 21 kg DM/ha per kg additional fertilizer N indicates that increasing N fertilization was economically profitable, because fertilizing costs are lower than the value of additional grass yield.

In addition to yield, also the concentration of most minerals and trace elements were positively correlated with N supply. Several factors may have contributed to the increased concentration of minerals including increased supply in the fertilizer in the case of at least N, P and K. Further, when the supply of growth limiting factors such as N is increased, the root development is enhanced resulting potentially to improved uptake of nutrients from the soil.

The supplies of Cu and Zn in feed are probably the most critical in dairy production, because of their multifunctional role in the metabolism of animals. The increased concentration of these trace elements is in accordance with individual fertilization studies. The actual increase in concentration between 0 and 100 kg N/ha was about 10 – 15 %.

Table 4. The effect of soil Cu, Zn and Mn content on the concentration of respective elements in grass.

| Soil status | | n | 1. cut | 2. cut | Average |
|---|---|---|---|---|---|
| 1 | Cu < 1.5 mg/l | 5 | 4.9 | 5.7 | 5.2 |
| 2 | Cu 1.5-2.7 mg/l | 62 | 6.5 | 7.0 | 6.8 |
| 3 | Cu 2.7-5.4 mg/l | 128 | 6.4 | 7.1 | 6.7 |
| 4 | Cu > 5 mg/l | 95 | 7.7 | 7.7 | 7.7 |
| 1 | Zn < 1 mg/l | 9 | 35.3 | 28.2 | 31.1 |
| 2 | Zn 1-1.5 mg/l | 7 | 34.3 | 32 | 33.3 |
| 3 | Zn 1.5-2 mg/l | 36 | 26.2 | 23.5 | 25.0 |
| 4 | Zn 2-6 mg/l | 187 | 32.8 | 31.2 | 32.3 |
| 5 | Zn > 6 mg/l | 42 | 33.0 | 40.4 | 37.1 |
| 1 | Mn < 6 mg/l | 15 | 52.0 | 55.2 | 51.9 |
| 2 | Mn 6-12 mg/l | 28 | 43.3 | 62.2 | 52.7 |
| 3 | Mn 12-25 mg/l | 187 | 50.8 | 66.9 | 58.0 |
| 4 | Mn 25-75 mg/l | 28 | 31.5 | 39.4 | 32.5 |

The effect of soil status on grass trace element contents was studied by ranking the observations according to the classification based on soil concentrations (Table 4). Copper content of grass increased with improved soil Cu status. There was also a trend towards higher contents of Zn and Mn with increased soil contents. These changes are in accordance with trace element fertilizing recommendations, since in the lowest status additional trace element fertilizing is recommended.

According to this data analysis, the N fertilizing level affects not only the grass yield but also the mineral and trace element content of harvested grass. Also there seems to be a correlation between soil status and grass mineral concentrations. Data analysis provides a useful tool to understand the effects of fertilizing on grass quality and results can be used to support practical recommendations.

# Nitrogen fixation by red clover as related to the supply of cobalt and molybdenum from some Norwegian soils

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**Abstract**
A field trial, a pot experiment and a survey in organically farmed leys were undertaken to investigate whether the N-fixation in red clover pastures in Norway was limited by a low supply of Co and/or Mo. Fertilization with Mo did not cause any higher production nor N-fixation, whereas the N-yield both from established clover leys and red clover grown in pots increased slightly after application of Co to many of the investigated soils. In the organically farmed leys there was a significant and positive correlation between the Co-content and the N-content of the red clover. As many of the investigated clover-soil systems were of those previously known to be very low in Co and Mo, and the gain in N-yield obtained by extra Co-supply was marginal, it is not likely that deficiency of these trace elements is a problem that deserves any great concern in legume based forage production systems in Norway.

**Note:**
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# A survey of manganese deficiency in Danish agriculture

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## Introduction

Manganese (Mn) deficiency has been well known and widespread in Danish soils for many years. There are no data which can reveal whether Mn deficiency has been an increasing problem over the years. The general impression from the advisory service is, however, that Mn deficiency causes more problems today than 20 years ago. The reason why Mn deficiency might have become more widespread can be an increasing area of susceptible winter cereals (winter wheat and winter barley) and a stop to the use of animal manure in September before the sowing of winter cereals. The area of winter barley and winter wheat has especially increased on soils susceptible to Mn deficiency, i.e., sandy soils or especially soils with a high content of organic matter. Mn deficiency is seen in a lot of different crops, but it is in winter wheat and especially in winter barley that the problem is significant. In many years Mn deficiency causes such serious damage to these crops in the late autumn and/or early spring that the crop must be re-sown for spring crops. Mn deficiency in spring-sown crops can cost a significant loss of yield but is normally easier to cure by application of foliar manganese products.

The traditional way to handle Mn deficiency is application of foliar Mn products either as prophylaxis treatments on fields known to be susceptible or treatments when the symptoms are determined in the field. Some susceptible fields are treated more than ten times a year with foliar applications. For winter barley and winter wheat one or two treatments in the autumn are recommended. Even the farmers are very aware of the problem as Mn deficiency has caused trouble for many years and recently in 2005 there were serious difficulties, especially in the northern part of Jutland.

For many years a number of ways to avoid manganese deficiency and to cure an observed deficiency have been investigated in trials organized by the Danish Agricultural Advisory Service. In this paper a review of the results of these trials will be given, along with the actual recommendations from DAAS.

## Susceptible soils

All Danish soils, with the possible exception of peat soils, have a natural high content of manganese with a typical variation from 150 to1750 kg manganese per ha in the upper soil layer (0-25 cm) corresponding to a concentration of 60-700 ppm Mn in the soil (Lamm,1964). Most of the Mn is fixed as Mn oxides and is not available for plants. Availability is controlled by the pH and the oxidation of the soil. The availability of manganese decreases 10 times if the pH increases by 0.1, and is therefore extremely influenced by pH.

In practice Mn deficiency is more pronounced on sandy soils, especially those with a high pH and/or a high content of organic matter, which means a more oxidized soil. Mn deficiency often occurs in special spots in the fields where the soil is sandier, has a higher content of organic matter and is looser and/or has a higher pH. A recent DAAS project has shown the variation in Mn concentration in the crop within the fields (data not published yet).

## Susceptible crops

Different crops have very different levels of susceptibility to Mn deficiency (Table 1) (Knudsen, 2001).

Table 1.

| High susceptibility | Medium susceptibility | Low susceptibility |
|---|---|---|
| Winter barley<br>Oat<br>Spring barley<br>Bean<br>Spinach<br>Sugar Beets | Winter wheat<br>Clover and alfalfa<br>Oil seed rape<br>Peas<br>Cabbage<br>Potatoes | Maize<br>Winter rye<br>Grass |

The combination of a susceptible crop on a susceptible soil must give problems. On very susceptible soils it is not recommended to grow winter barley because it can be uncertain if it is possible to avoid Mn deficiency in this crop. An alternative can be to replace the winter barley with winter rye, which is much more tolerant of Mn deficiency than winter barley.

**Varieties**

Different varieties of cultivars differ in their susceptibility to Mn deficiency (*Graham, 1988*). Since 2001, DAAC, together with the Royal Veterinary and Agricultural University (KVL), have carried out intensive work in ranking varieties of winter barley according to their susceptibility to Mn deficiency. Different varieties have been tested without and with foliar application of Mn on soils with low manganese supply. A summary of the results is shown in table 2 as the yield response to foliar application of 3-5 times 2.5 kg manganese sulphate per ha for different varieties.

Table 2. Yield respons for spraying manganese in winter barley varieties, 2002 – 2004. (N23) (Pedersen, 2004).

| | Yield response for spraying manganese sulphate autumn and springtime, dt per ha | | |
|---|---|---|---|
| **Winter barley** | **2004** | **2003** | **2002** |
| *Number of trials* | *3* | *2* | *2* |
| Mixture | 11.3 | 4.8 | 5.1 |
| Himallaya | 21.2 | | |
| Ludo | 20.5 | 6.6 | 1.1 |
| Cleopatra | 15.5 | 5.8 | 14.5 |
| Carola | 11.0 | 6.3 | 0.2 |
| Vanessa | 22.6 | 4.0 | 6.7 |
| Chess | 21.8 | | |
| Escape | 21.8 | 5.2 | 10.4 |
| Antonia | 27.4 | 5.4 | 14.5 |
| Menhir | - | 3.4 | 0.2 |
| Clara | - | 3.0 | |
| Hanna | - | - | 11.7 |
| Siberia | - | - | -1.2 |
| Platine | - | - | 10.4 |

Table 2 shows that there was a significant difference in 2002 and 2004, when there was a serious manganese deficiency in the trials. It is also clear that no varieties are so tolerant of a low manganese supply from the soil that there is no need for foliar application. Hebbern (2005) gives a more detailed description of the results and supplemental scientific studies done by KVL.

The current recommendation from DAAS is that, if the farmer wants to grow winter barley on manganese susceptible soils, he must choose the variety Carola (6 rowed variety), which both in 2002 and 2004 performed with the lowest response to manganese application and had a high yield.

**Manganese and seed**

From studies in Australia it has been reported that manganese content in seed can have a significant influence on the susceptibility of Mn deficiency because the development of the roots right after germination can be limited by a low Mn content in the seed (Graham, 1988). In trials in Durum Wheat it was found that the growth of roots and shoots in a Mn inefficient variety was increased with increasing Mn content in the seed from 0.4 to 1.0 yg per seed, corresponding to 7 to 18 ppm in the seed. Screening of the Mn content in 26 samples of winter barley seed in Denmark showed an average concentration of manganese at 13 ppm with a variation from 8 ppm to 19 ppm (Pedersen, 2001). Based on these results from Australia the content of Mn in seed could be a significant factor in Mn susceptibility.

DAAS have tested the hypothesis of the influence of manganese content in seed in trials in 2000, 2002 and 2004. In two trials in 2000 and 2002 the same variety with a low (9-10 ppm Mn) and a high (18-20 ppm) concentration of Mn in the seed was grown on fields with a low supply of Mn from the soil, both without and with foliar application of $MnSO_4$. No differences between low and high Mn content in seed were observed in Mn deficiency in the crop or in yield response for foliar application of Mn (Pedersen, 2002). In 2003 seed of winter barley was produced from the same field with a low and a high Mn content in seed by foliar application of Mn to a part of the field at flowering. Seed with low (11 ppm Mn) and with high (16 ppm Mn) manganese content was tested in 2004 without and with foliar application of Mn. No significant differences were found and the effect of Mn content in the seed was totally overruled by foliar application in the autumn (Pedersen, 2004).

Different products (Cutonic Mn-primer, Cillus Mn-bejdse) for seed treatments have also been tested at DAAS. In the trials an effect of the seed treatment is observed on the Mn deficiency in the autumn, but the seed treatment must be supplemented by foliar application of Mn in the autumn (Pedersen, 2004). Therefore seed treatment with Mn products has not been a common practice in Denmark.

**Fertilizer types and Mn deficiency**

Application of fertilizer or organic manure can change the pH in the soil. The type of fertilizer, application time and form therefore have a significant influence on the Mn supply from the soil.

Ammonium sulphate compared to ammonium nitrate and urea gives a significantly higher manganese uptake in both spring- and autumn-sown crops (Skriver,1990, Schnug,1982). Using ammonium sulphate compared to calcium ammonium nitrate means higher manganese uptake than the use of fertilizers with manganese (Schnug,1982).

Placement of a nitrogen fertilizer results in a better Mn supply from the soil compared to broadcasting of the fertilizer because the pH is lower for a longer time around the string of fertilizer placements (Goldberg, 1982). Petersen (2001) have shown that placement of slurry gives a better uptake of Mn compared to broadcast application.

The problem of practical use of these results in Danish agriculture is that the most serious problem with Mn deficiency occurs in winter cereals, which are without fertilizer application in the autumn. Application of nitrogen fertilizer at that time is regarded as giving a high risk of leaching of nitrogen. Fourteen trials at DAAS in all in winter barley and winter wheat from 2000 to 2004 show that the manganese content in grain at harvest was 2-3 ppm higher in plots where 60 kg N per ha were given as ammonium sulphate compared to NS 24-8 or NS 21-7 with 0.8 pct. Mn. In all of the trials additional nitrogen was given in the form of pig slurry. In 5 of the trials there were problems with manganese deficiency and but a significant yield response was obtained by using ammonium sulphate compared to NS 21-7 (Pedersen, 2004).

In general DAAS recommend use of placement of fertilizer together with establishing of spring-sown crops to improve nitrogen utilization. This recommendation is even more important on fields with a low soil Mn supply because of the improvement of Mn availability. Placement of fertilizer also reduces the problems of weed control. In winter cereal use of ammonium sulphate is only recommended if there is need for a permanent reduction of the pH of the field.

## Foliar application of Mn

Use of foliar application of Mn products is very common in Denmark. At DAAS we have over the years tested a considerable number of different types of products and formulations. The results can be summarized as the effect of the different products per gram of applied Mn. We have not been able to find any improved effect of, e.g., manganese nitrate, manganese carbonate, manganese chelates compared to the traditional and cheaper manganese sulphate.

Manganese sulphate in powder form (32 pct. Mn) is normally added with an additive (e.g. Lissapol) to improve its uptake. Some farmers have technical problems with a solution of the product and prefer a liquid formulation. A list of typical foliar products in Denmark is shown in table 3.

**Table 3.** Examples of manganese products for foliar application

| Example of product for the type of formulation | Type | Content | Price for the product, kr/unit | Dosage and price pr. ha by 100 g Nm pr ha [1)] | Dosage and price pr. ha by 400g Mn pr. Ha[2)] | Price pr. kg Mn |
|---|---|---|---|---|---|---|
| Manganese sulfphate (powder ) | Manganese sulphate | 320 g pr.kg | 8 kr. pr. kg | 0,3 kg pr. ha [3) + 4)] 3 kr. pr. ha | 1,3 kg pr. Ha [3) + 4)] 10 kr. pr ha | kr. 25 |
| DDP Manganase | Manganes sulphate+ manganes chlorid | 330 g pr.kg | 85 kr. pr. kg | 0,3 kg /ha 26 kr. pr. ha | 1,3 kg/ha 104 kr. pr. Ha | kr. 260 |
| CarboMan 500 | Manganes carbonate | 500 g pr l | 49 kr. pr. l | 0,2 l pr. ha 10 kr. pr. ha | 0,8 l pr. ha 40 kr. pr. ha | kr. 100 |
| Mantrac 500 | Manganes carbonate | 500 g pr l | 32 kr. pr.l | 0,2 l pr. ha 6 kr. pr. ha | 0,8 l pr. ha 25 kr. pr. ha | kr. 64 |
| NitraMan | Manganes nitrate | 235 g pr l | 29 kr. pr. l | 0,4 l pr. ha 13 kr. pr. ha | 1,7 l pr. ha 50 kr. pr.ha | kr. 125 |
| Mangan 180 | Manganes nitrate | 180 g pr l | 20 kr. pr. l | 0,6 l pr. ha 11 kr. pr. ha | 2,4 l pr. ha 45 kr. pr. ha | kr. 110 |

1) Only profylaxis treatment where no manganese deficiency normally not occur.
2) Dosage per application where manganese deficiency is diagnostified or on field with low manganese supply from soil
3) According to the low price normally 2-3 kg of manganese sulphate is used per ha.
4) There must be an additive.

In winter barley and winter wheat grown on fields with a low soil Mn supply one or two applications of foliar manganese are recommended in autumn and again at the beginning of growth in the spring.

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# Trace elements in crops from Swedish and Icelandic long-term experiments

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## Introduction

Agricultural crops provide trace elements necessary for the metabolism of farm animals and humans. In this sense, agriculture can be considered as a nutrient supplier. Agricultural fields have been fertilized with macronutrients for decades but fertilization with trace elements has been limited. The question arises if increasing yields and limited addition of trace elements with inorganic fertilizers have led to decreasing trace element contents of plants. The aim of this investigation was to test if concentrations of trace elements in wheat from Sweden and grass from Iceland, grown in long-term field experiments receiving inorganic macronutrients only, have changed during a period of 30 years.

## Materials and methods

Archived wheat kernels from 10 sites in Sweden and archived grass from 4 sites (6 experiments) in Iceland fertilized with inorganic macronutrients over 4 decades, were analyzed for 11 trace elements. Samples from three early and three late years (average interval around 30 years) were chosen. Icelandic results are from 1$^{st}$ cut only although the plots were cut twice most of the years.

After digestion in concentrated nitric acid, trace elements were determined on an inductively coupled plasma-mass spectrometer (Elan 6100 ICP-MS, Perkin Elmer SCIEX instruments). The detection limit for trace elements was 0.04 µg kg$^{-1}$. Dry matter contents in crop samples were determined and metal concentrations are given in mg per kg crop dry matter (DM).

Removal of trace element by crops was calculated using yield data. Statistical analyses were done in REML models with years as a random component. Locations were also treated as a random factor in the wheat data from Sweden whereas in the Icelandic material more data were available from each location so that interactions with locations as well as treatment effects (N-fertilizers affecting soil reaction at two locations) could be tested. The tests apply to comparisons of adjusted means which usually differ slightly from the means tabulated in the Results section. In cases with large differences between locations or skew distribution methods based on the normal distribution could not be applied. In these situations the data were split in groups or the Mann-Whittney test was used, respectively. Alternatively frequency distributions were compared in the case of skew data. Occasional extreme high values, probably due to contamination of the sample, were eliminated from the analyses by the REML model (Fe and Pb in Iceland, Pb in Sweden, 1 or 2 values) but this is not needed for the Mann-Whittney test.

## Results and discussion

Mean concentrations of some elements decreased over time, some increased and others remained constant (Table 1). For some elements there was a great variation between locations or soil types. The average increase for few elements was unexpected and could be explained with very small, unknown amounts in the fertilizers or changes in pH with time. In the Swedish soil fertility studies there was a slight decrease of soil pH values over time but lime was added to maintain values between 6 and 7. Some sites had high initial pH values (>7) that decreased to below 7. Thus, the effect of soil pH on trace element solubility was at minimum. In Iceland, the pH changes varied between plots depending on type of fertilizer. However the general trend in the Icelandic plots was a small decrease in pH with time, except in those plots that got fertilizer with Ca.

A clear yield increase over time was measured in the Swedish experiments while in Iceland yields were slightly lower in the second period. Using yield data to calculate the removal of trace element (Table 2) showed that removal trends differed from concentrations trends (Table 1). Despite decreasing concentration of Zn, Fe, Cu, Cd in crops, the uptake remained constant or increased. A feasible explanation is that the decreasing concentrations were caused by dilution due to higher yields. Of these four elements, Cu was significantly negatively correlated with yield within locations and periods, which clearly supports the hypothesis that dilution caused lower concentrations. Zn also showed the same trend within periods but it was weaker. Other elements that were negatively correlated with yield within periods are Mo and Cd also indicating the dilution effect for these elements. No significant change in the removal of trace elements were found in the Icelandic results with the exception of Cr, which increased with increasing yield.

*Molybdenum* In Sweden, the average values of Mo increased at all location except one with very low values. In Iceland, Mo increased on sandy soil from 0.09 to 0.47 mg/kg DM but not in other soil types. The Mo content of plants is normally less than 1 mg/kg and Mo-deficient plants contain less than 0.2 mg/kg (Havlin et al., 2005). The solubility of molybdenum, unlike other micronutrients, decreases at lower pH values and higher concentrations in crops cannot be explained through lower soil pH values. In the Swedish experiments, the removal of Mo through crops increased by 200 % (Table 2) while it remained constant in Iceland.

*Zinc* There was a significant decrease of Zn concentration in crops at six Swedish sites and no change at four sites with the highest values. Zn-concentrations in winter wheat (32 mg/kg) were above the critical limit of 10 mg/kg and similar to those reported by Eriksson et al. (2000). However, the removal through crops increased over time in the Swedish experiments (Table 2). No significant change was measured in the grass crops in Iceland but the trend was towards decreasing content and uptake.

*Nickel* Ni-concentrations did not change over time in Swedish or Icelandic crops but Ni-concentrations were about ten times higher in the Icelandic crops. Nickel in plants normally range from 0.1 to 1 mg/kg. Nickel was found to be an essential plant nutrient in 1987, when it was shown that a limit concentration of 0.1 mg/kg was necessary for the germination of seeds (Brown et al. 1987).

*Iron* Swedish data indicate significantly lower Fe-contents (23.5 mg/kg) in crops sampled after 3 decades than those sampled early (31.8 mg/kg) whereas no difference was detected in crops from Iceland (mean 140 mg/kg). Furthermore, concentrations in Swedish grain are below the critical limit (<50 mg/kg). Data in Table 2 indicate that the uptake of Fe by crops from Swedish sites remained constant but due to increasing yields over time (data not shown), concentrations decreased. Soil organic matter an important factor affecting iron solubility, had not decreased over time at the sites (Carlgren & Mattsson, 2001).

Table 1. Average trace element concentrations (mg kg$^{-1}$ DM) in wheat (Sweden) and grass (Iceland) from early and late experimental years (30-40 years interval). Ten Swedish and four Icelandic sites; 58-60 observations in each country.

| | **Sweden** | | | **Iceland** | | |
|---|---|---|---|---|---|---|
| **Element** | Early | Late | Significance | Early | Late | Significance |
| Mo | 0.45 | 1.18 | *** | 0.37 | 0.41 | * (sandy soil) |
| Zn | 36.1 | 31.9 | * (some locations) | 40.1 | 37.0 | ns |
| Ni | 0.124 | 0.134 | ns | 1.15 | 1.17 | ns |
| Fe | 31.8 | 23.5 | ** | 150.2 | 137.8 | ns |
| Se | 0.024 | 0.023 | ns | 0.058 | 0.067 | * (sandy soil) |
| Mn | 25.48 | 31.26 | * | 128.7 | 146.2 | *(sandy and bog soil) |
| Cu | 3.57 | 3.12 | * | 8.24 | 6.83 | ** |
| Co | below detection limit | | | 0.168 | 0.175 | ns |
| Cr | 0.014 | 0.006 | *** | 0.17 | 0.29 | *** |
| Cd | 0.069 | 0.047 | *** | 0.090 | 0.126 | *(sandy and bog soil) |
| Pb | 0.079 | 0.023 | *** | 0.44 | 0.07 | *** |

Table 2. Average uptake (g ha$^{-1}$) of trace elements by winter wheat grains (Sweden) and grass (Iceland) at early and late experimental years (30-40 years interval).

| | **Sweden** | | | **Iceland** | | |
|---|---|---|---|---|---|---|
| **Element** | Early | Late | Significance | Early | Late | Significance |
| Mo | 1.94 | 5.99 | *** | 1.62 | 1.52 | ns |
| Zn | 143.5 | 180.1 | *** | 170.6 | 134.1 | * (one location) |
| Ni | 0.293 | 0.415 | * | 4.85 | 4.13 | ns |
| Fe | 128.1 | 135.3 | ns | 650.7 | 505.1 | ns |
| Se | 0.11 | 0.16 | ns | 0.25 | 0.24 | ns |
| Mn | 106.1 | 178.4 | *** | 527.9 | 501.8 | ns |
| Cu | 14.4 | 17.7 | *** | 35.6 | 24.9 | ** |
| Co | below detection limit | | | 0.717 | 0.625 | ns |
| Cr | 0.058 | 0.030 | * | 0.86 | 1.27 | ** |
| Cd | 0.285 | 0.274 | ns | 0.388 | 0.461 | ns |
| Pb | 0.31 | 0.13 | *** | 1.68 | 0.26 | *** |

*Selenium* Se-concentrations in Swedish crops sampled north of Skåne were below the detection limit (0.04 µg/kg). The highest Swedish values are all from one location, Fjärdingslöv in Skåne (0.024 mg/kg). In Iceland, there was a significant increase in Se-concentrations in crops over time at a sandy site from 0.024 to 0.075 but not in other soil types. The soil organic matter content has increased at the sandy site. The Se-levels in Swedish samples are below the
minimum range of concentration, 0.03-0.1 mg/kg DM required for human health and optimal performance of grazing ruminants (O'Dell & Sunde, 1997).

*Manganese* A significant increase in Mn-levels in crops over time was found in all Swedish and in Icelandic samples from sandy and bog sites. Removal through crops was almost twice as high after 3 decades. The main reason may be somewhat lower pH values, which increases the solubility of Mn. Manganse levels in crops are far above the critical limit of 10 mg/kg.

*Copper* Cu-concentrations in crops decreased significantly over time in the Swedish samples but variations between locations were large. Also in the Icelandic samples a significant decrease with time was found. Still, concentrations are far above the deficiency level of 2-5 mg/kg.

*Cobalt* Co-contents in Swedish crop samples were below the detection limit. There was no change in concentration or removal over time in the Icelandic samples.

*Chromium* Concentrations of Cr in Swedish winter wheat decreased by more than 50% but increased in Icelandic samples.

*Cadmium* Concentrations of Cd decreased significantly over time in Swedish wheat but the total uptake of Cd by winter wheat remained relatively constant over time. Carlgren and Mattsson (2001) found decreasing Cd concentrations in all treatments in the soil fertility experiments. The decline in crop Cd concentrations seems to be the result of dilution through higher yields. The use of P fertilizers low in Cd and reduced atmospheric fallout may not necessarily be the main reasons for lower Cd concentrations in crops. In Icelandic crop samples from sandy and bog soils, concentration and amounts of Cd taken up by crops increased over time.

*Lead* Pb-concentrations in crops and amounts removed decreased drastically over time in all samples. The exclusion of lead as an amendment to petrol and other measures reducing the atmospheric fallout of Pb seem to be the most probable reasons.

**Conclusions**

The drastic decrease in Cr and the more subtle decline in Fe, Zn and Cu in Swedish winter wheat and Cu in Icelandic grass require attention. Very low levels of Fe, Se and Co in Swedish samples need to be recognized both regarding the sufficiency for crops (Fe) and for human diets (Se and Co). Declining trends in Pb uptake by crops over time indicate that the presence of non-wanted metals in crops can be controlled within decades.

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# Trace elements in animal nutrition

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**Introduction**
All living organisms require inorganic elements, or minerals, for their life processes. As such, all feeds and animal tissues contain minerals in widely varying amounts and chemical forms. Minerals are classified in a number of ways. Often minerals that are needed in relatively large amounts are referred to as macrominerals whereas minerals that are needed in very small amounts are denoted microminerals or trace elements. Seven minerals are macrominerals and 22 can be referred to as trace elements (McDowell, 2003).

Table 1. Essential minerals

| Macrominerals: | Trace minerals: | Newer trace elements: | |
|---|---|---|---|
| Calcium (Ca) | Cobalt (Co) | Aluminium (Al) | Germanium (Ge) |
| Phosphorus (P) | Chromium (Cr) | Boron (B) | Lithium (Li) |
| Potassium (K) | Iodine (I) | Fluorine (F) | Rubidium (Rb) |
| Sodium (Na) | Molybdenum (Mo) | Lead (Pb) | Tin (Sn) |
| Sulfur (S) | Zinc (Zn) | Nickel (Ni) | |
| Magnesium (Mg) | Copper (Cu) | Silicon (Si) | |
| Chlorine (Cl) | Iron (Fe) | Vanadium (V) | |
| | Manganese (Mn) | Arsenic (As) | |
| | Selenium (Se) | Bromine (Br) | |

An element is defined essential when it is required to support adequate growth, reproduction, and health when all other nutrients are optimal. However, the listing of minerals as essential is often difficult and sometimes tentative. The proof that a mineral is essential rests upon experiments (with one or more species). All the mentioned minerals have not been tested in all species, but it is highly probable that there are some exceptions to the need for all of them by higher animals (McDowell, 2003). Currently, Cr, Co, Cu, I, Fe, Mn, Mo, Se and Zn are classified as important trace elements for livestock.

**Functions of the minerals**
This presentations deal with the trace elements but the metabolism, functions and utilization of the trace elements are often closely connected to the macrominerals. As such, many statements also refer to the macrominerals. In contrast to many other substances the minerals cannot be synthesized in the body but have to be provided via the feed. In the body, minerals have four main functions: (i) structural, (ii) physiological, (iii) catalytic, and (iv) hormonal or regulatory. Many of the trace elements are especially involved in the latter two tasks. The functions of minerals are interrelated and balanced against each other and most often cannot be considered as single elements with independent and self-sufficient roles in the body processes. This may complicate the interpretations of results obtained in mineral experiments and the comparison of results in different experiments.

The concentrations of minerals in the body must be maintained within rather narrow limits if the functional and structural integrity of the tissues is to be safe and the performance of the animals are to remain unimpaired. Continued ingestion of diets that are deficient, imbalanced or excessively high in a mineral induces changes in the form or concentration of that mineral in the body tissues and fluids, so that it falls below or rises above the tolerable limits (Underwood and Suttle, 2001). In such circumstances, biochemical lesions develop, physiological functions are affected adversely and structural disorders may arise, in ways that vary with the element, the degree and duration of the dietary deficiency and oversupply. The reactions also may vary between species of animals and be dependent on age and sex of the animals. However, the homeostatic mechanisms in the body may delay or minimize the onset of such diet-induced changes. Ultimate prevention of the changes requires that the animals be supplied with a diet that is palatable and non-toxic and which contains the required minerals – as well as other nutrients and energy – in adequate available amounts (Underwood and Suttle, 2001).

**Trace element nutrition**

Large numbers of livestock in many parts of the world consume diets that do not meet their requirements and nutritional disorders arise. However, in this part of the world the insufficiencies of the intrinsic mineral content in feedstuffs are ameliorated by mineral supplementation. As such, the incidence and severity of mineral malnutrition in livestock is not often seen in livestock production and for long time it has been common practice add rather too much than too little – just to be safe. Now this practice is changing because there is increasing focus on the excretion of minerals, especially the heavy metals like copper and zinc in intensive pig production. Furthermore, the provision of extra minerals beyond the animal's requirements is economically wasteful, confers no additional benefit on the animal and can be harmful.

As mentioned, livestock animals get a high proportion of their minerals from the feedstuffs and forages that they consume. For this reason, the factors that determine the mineral content of the vegetative parts of plants and plant seeds are the factors that are important when evaluating the mineral intakes by livestock. As an example, in some areas the soil is low in selenium and this gives rise to crops (seeds and forages) low in selenium. Formerly, this induced selenium deficiency in farm animals but this is ameliorated either by supplementing selenium to the diets or by spraying selenium directly onto the crops. This paper will not focus on how the mineral content in feedstuffs and forages vary and by which means the mineral content can be manipulated. However, it is well known that the mineral content may vary quite a lot.

**Trace element availability**

The evaluation of feeds and feed supplements as sources of minerals depends not only on the total trace element content or concentration but also on how much can be absorbed from the gut and used by the animals' cells and tissue. This may depend on the species and age of the animals. Furthermore it is influenced by e.g. the chemical form in which the mineral is ingested, the amounts and forms of other dietary components with which the mineral interacts, the intake of the mineral relative to need etc.

Assessment of the physiological availability of trace elements in feeds and mineral supplements presents a lot of difficulties like the risk of contaminations. Ammerman has made a comprehensive review of the different methods that have been used to determine the bioavailability of minerals (Ammerman et al., 1995). Absorption of the mineral may provide an estimate of its bioavailability. The mineral must be absorbed from the gastrointestinal tract, and the assumption is generally made that, once absorbed, the element is available for storage or for use in various physiological processes by the animal. Absorption, however, cannot always be equated to bioavailability (Ammerman et al., 1995). When mineral elements, such as sulfur and selenium, are given to an animal in the form of sulfur-containing amino acids, absorption and metabolism within the body may differ from that which occurs when other forms of the element are provided. Selenium as sodium selenite and selenomethionine, for example, can be absorbed and metabolized by different pathways, and comparisons of the bioavailability of the element based on absorption or tissue deposition may be misleading (Ammerman et al., 1995). Absorption studies have mainly been conducted with macrominerals but have also been used in a few studies with trace elements. Often the results are presented relative to a standard source but a few studies on zinc have recently been presented. Over the years a lot of different *in vitro* techniques have been used to estimate the degree to which the mineral source would be utilized by animals. However, generally *in vitro* solubility is a poor indicator of *in vivo* bioavailability (Ammerman et al., 1995). Measures have been undertaken by use of stabile or radioactive isotopes depending on the studied element.

### Trace element requirement and status

Recommendations on (trace) mineral needs in livestock animals are often given as minimal requirements with an extra mount serving as safety margin. These safety margins are included to compensate for e.g. differences in bioavailability, differences between results obtained in different experiments, different feeding regimes, diets etc. Often the requirement is defined on the basis of doses response experiments using relevant response characteristics for the mineral in question. The response characteristics may include productivity (growth, feed intake, reproductive performance), health (immune responses, health parameters e.g. diarrhea, bone development), blood parameters and other physiological responses. However, for many years a major goal in mineral research has been to discover or develop simple and accurate biochemical measurements of the status of animals for the minerals in which there are important practical problems. However, this has not been very successful.

### Zinc – an essential trace element

Many animal diets require supplementation with zinc because of either low dietary levels or the presence of dietary factors that decrease the bioavailability of the element. The critical importance of added zinc for livestock animals was shown in 1955 when it was demonstrated that parakeratosis, a condition being observed in pigs, was caused by inadequate zinc. It was the common practice to feed high calcium levels along with plant protein containing phytate. This apparently reduced the bioavailability of dietary zinc to the point that a severe deficiency of the trace element occurred. This was also found in poultry and later observations in ruminants showed that zinc deficiency can occur under grazing conditions in some areas of the world. Zinc is without doubt a very essential trace element and several comprehensive reviews describing the physiological functions, metabolisms and utilization by humans and animals have been published (Hambidge et al., 1985; Cousins, 1995).

### Concluding remarks

The presentation will discuss the matters presented in this abstract by including relevant examples and experiments conducted mainly with livestock animals and trace elements. Furthermore, actual problems in trace element nutrition will be addressed.

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# Selenium and GPX activity in blood from pregnant and non-pregnant ewes and selenium in forage on sheep farms of various scrapie categories in Iceland

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## Introduction

Each year scrapie is found sporadically on a few farms in Iceland, mostly in four or five previously affected areas in southern and northern parts of the country. The sporadic occurrance of scrapie indicates a link to some environmental factor(s) that may cause or are a risk for the development of clinical scrapie. Previous research by the authors suggests that high concentration of manganese in forage, or high manganese/copper ratio in forage, may have preventive effect on recurrence of scrapie (Jóhannesson *et al*. 2004a). The aim of this study was to study whether an imbalance of selenium (Se) or the activity of glutathione peroxidase (GPX) in blood of sheep might be connected with occurrence of scrapie.

## Materials and methods

Forage samples (round bale silage and in a few cases hay, in total 88), from the summer harvest of 2002 and/or 2003 and blood samples (in total 125), were collected ( from 2-5 year old ewes in autumn 2002 – autumn 2003) on 17 sheep farms for analysis of selenium and glutathione peroxidase (GPX) activity. On the scrapie-free and scrapie-prone farms (see below), blood samples were collected both from non-pregnant ewes in the autumn and from pregnant ewes in the spring, as pregnancy and feeding in stables for prolonged periods may affect blood selenium levels and GPX activity. On scrapie-afflicted farms blood samples were collected at the outbreak of scrapie (when the ewes were either pregnant or non-pregnant) and the sheep flocks were then culled shortly after the diagnosis was made. Farms were divided into three categories. *Scrapie- free*: Farms never afflicted by scrapie, or afflicted and restocked with healthy sheep prior to 1960. *Scrapie-prone*: Farms afflicted by scrapie after 1980 and afterwards restocked with healthy sheep. *Scrapie-afflicted*: Farms where scrapie was diagnosed during the experimental period (summer 2002 – March 2004). The farms are all located in four scrapie affected counties, fourteen of them being in the Vatnsdalur-Vídidalur area in northern Iceland. Selenium was determined in the samples by hydride generation atomic absorption (Perkin Elmer MHS-20) and GPX activity with a spectrophotometric assay using a UNIFAST 3 analyzer. Furthermore, a questionnaire was sent to 20 veterinarians around the country. They were asked about how often signs of selenium deficiency, especially in newborn lambs (heifers, foals) were observed in their areas. Inquiry was also made about what preventive measures, such as injection of lambs with selenite, the use of special feeding salts or fertilizers, were taken in cases of suspected selenium deficiency.

## Results

Selenium concentration was low or very low in almost all forage samples, or in the range 15-25 μg $kg^{-1}$. No statistically significant difference was found between selenium concentration in forage from farms in different categories. Selenium concentration in blood of ewes was generally above 100 ng $ml^{-1}$ in non-pregnant ewes, but below 100 ng $ml^{-1}$ in pregnant ewes. Information gathered from 20 veterinarians all over the country revealed that selenium deficiency in lambs is widespread in Iceland. In ewes on eight scrapie-free and scrapie-prone farms the selenium concentration in whole blood declined sharply from the non-pregnant state in autumn to the pregnant state the following spring. The GPX activity declined concomitantly in whole blood, but to a lesser degree, resulting in a significantly higher GPX/Se ratio in the pregnant state and a far less significant correlation between Se concentration and GPX activity than while the ewes were not pregnant. Ewes on scrapie-free farms had a significantly higher concentration of Se and higher GPX activity in the non-pregnant state than non-pregnant ewes on scrapie-prone farms but this was not true of the pregnant state. Se concentration and GPX activity in non-pregnant ewes on 4 scrapie-afflicted farms did not differ significantly from neither non-pregnant ewes on scrapie-free farms nor scrapie-prone farms. In other experiments GPX activity was found significantly lower in blood of ewes, whether non-pregnant or pregnant, on scrapie-prone and scrapie-afflicted farms than in blood of ewes on scrapie-free farms. Low GPX activity may thus, and seemingly independent of selenium concentration in blood, be somehow connected to scrapie (unpublished results).

## Discussion and conclusions

It is generally accepted that feeding livestock forage with selenium below 100 μg $kg^{-1}$ causes risk for deficiency symptoms (Adriano 2001). The selenium concentration in forage in the present study was very low. Others have previously found low amounts of selenium in samples of Icelandic hay or herbage (Eiríksdóttir *et al.* 1981, Símonarson *et al.* 1984, Purdey 2000). It is also the general consensus that selenium concentration in blood of sheep above 100 ng $ml^{-1}$ is sufficient, levels between 50 and 100 ng $ml^{-1}$ are marginally sufficient and levels below 50 ng $ml^{-1}$ deficient (Hamliri et al. 1990, Neve 1991). The ewes in the present study were thus in sufficient selenium status when non-pregnant, but only marginally sufficient when pregnant. Taken together with the information given by the 20 veterinarians located all over the country it must be assumed that selenium deficiency is a fairly ubiquitous phenomenon in sheep Iceland (Jóhannesson *et al.* 2004b).

The three main conclusions of this research are: First the low selenium concentration in forage on farms in all categories, and probably all over the country, is not likely to be directly connected to sporadic occurrence of clinical scrapie. Second it is controversial whether ewes on scrapie-prone or scrapie-afflicted farms may differ in selenium status from ewes on scrapie-free farms. Third it is necessary to define the status of the ewes, non-pregnant, pregnant or otherwise, at the time of sampling for determination of selenium concentration or GPX activity in blood. This last point applies equally to studies relevant to other conditions as well as to scrapie.

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# Variation in hepatic copper accumulation in sheep – seasonal and genetic effects

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**Introduction**

In Norway, in contrast to most other countries, chronic copper poisoning is an endemic disease, principally affecting adult ewes in the autumn, after their return from mountain or woodland pastures. On a national scale, the prevalence is low, but geographical and flock-to-flock variation is large. The disease is practically non-existing along the coast, but well known in several inland districts. Also within affected flocks the prevalence may wax and wean. In some flocks, a yearly death rate of 1-3 % of adult ewes over a five year period has been observed (*Sivertsen & Wie* 1996).

In the 70's and 80's, some of the background for the geographical differences in occurrence was clarified. Surveys of hepatic copper levels in normal adult sheep at slaughter showed more than 150 mg/ kg liver tissue (ww) in up to 54 % of ewes from inland areas, but in less than 2 % of ewes from the west coast (*Frøslie* 1980). Studies of mineral composition of forage and pasture indicated that the main reason for the copper accumulation in inland sheep is low molybdenum levels in inland grass and mountain pasture plants, leading to very high copper/ molybdenum ratios (*Frøslie & Norheim* 1983, *Garmo et al.* 1986).

These results naturally raised the question why the clinical disease is not more prevalent than it seems to be. On this background, our studies have concentrated on three flocks in an inland mountain area, with substantial toxicity problems.

**Materials and methods**

To attain general knowledge on the situation in the flocks, liver samples for copper analysis were collected at slaughter. In the worst affected flock, samples were collected over a period of 9 years.

To study the accumulation in individual sheep over time, we developed a method to analyze copper content in liver biopsies taken out under field conditions, and followed the hepatic copper levels in a number of sheep in the worst affected flock through two years. Biopsies (two each time) were taken with Bard Biopty® equipment after ultrasound scanning. The biopsies were frozen immediately in liquid nitrogen and stored in small closed plastic tubes at – 70º until analysis. Copper content was determined by atomic absorption spectroscopy after oxidative digestion. Altogether, nearly 300 biopsies were analyzed, from 36 sheep. One ewe was lost, due to fatal bleeding from a hepatic vessel. The biopsies were taken in December, March, June and October. We achieved complete sets of four biopsies from 14 ewes each year, and complete sets through both years from 4 ewes (Fig. 1).

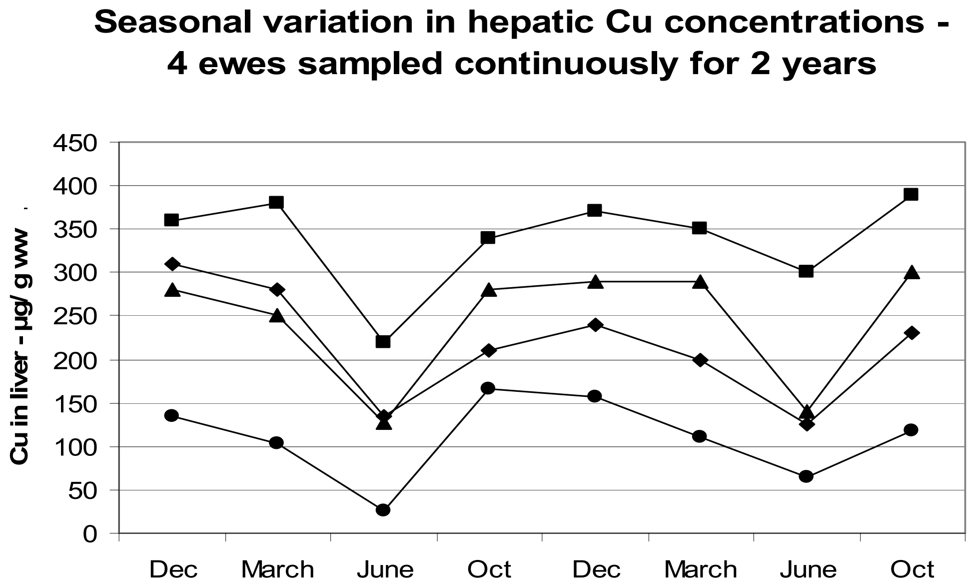
To study possible genetic effects, the owner's breeding records for the same flock in the relevant years were combined with the analytical data.

**Results and discussion**

The analyses of liver samples at slaughter confirmed that the differences in toxicity problems tend to be related to the average hepatic copper concentrations also at flock level. In the year of highest poisoning prevalence, adult sheep in the worst affected flock had a mean hepatic copper level of 465 mg/ kg ww at slaughter (*Sivertsen & Wie* 1996).

From the biopsy studies, two results emerged clearly: First, there was a systematic seasonal variation in hepatic copper content. The levels changed only moderately from December to March, fell substantially from March to June, but rose again to high levels in the mountain pasture season from June to October (Fig.1). The fall in hepatic copper in the spring may possibly be related to transfer of copper to the lambs in pregnancy, while the strong rise in the summer confirms the importance of the mountain pastures in copper accumulation. If our results are representative for the situation in other affected flocks, the variation in hepatic copper levels may explain the seasonal occurrence of chronic copper poisoning in Norway.

Figure 1:

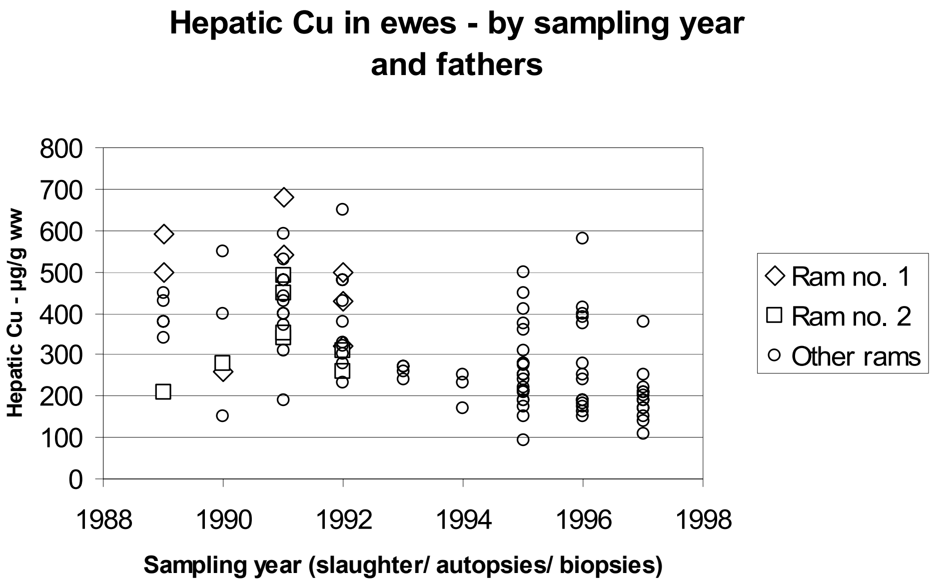



Secondly, as can also be seen from Fig.1, there were large individual differences between ewes, and these differences were stable through both years.

The results of the slaughter, autopsy and biopsy analyses collected in the flock showed a gradual decline in the hepatic copper levels in the last 7 years of the study period (Fig. 2). The stable differences in hepatic Cu levels between the sampled ewes in the biopsy study naturally raised the question of a possible genetic variation in copper accumulation between individual sheep. In our analysis of the breeding records, we could identify 54 mother-daughter pairs with data on hepatic copper levels in the autumn. There was a weak correlation between mother and daughter values. When analyzed on log transformed values, the correlation was significant ($p<0.05$). Comparison of half-sibling groups that were daughters of different rams was complicated by the breeding system in the flock. The rams were changed from year to year, and daughters of each ram tended to be slaughtered within the same years. Therefore, the possible effect of different rams was entangled with the gradual change from year to year. However, between two of the rams that had many daughters slaughtered in the same four years, a difference between hepatic copper levels in the daughters was observed (Fig. 2). When corrected for average level each year, the difference was significant ($p<0.01$).

These results may indicate that some of the individual, flock-to-flock and temporal variation in hepatic copper accumulation in sheep in Norway does have a genetic component. Still, much of the variation seems to be influenced by factors that we still do not understand.

Figure 2:





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# Trace element status of soil and organically grown herbage in relation to animal requirements

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## Introduction

Ruminants depend on an adequate supply of zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), molybdenum (Mo), cobalt (Co), selenium (Se) and vitamin E. A transition from conventional to organic farming practise may change the content and availability of soil trace elements and thereby the herbage and feed trace element composition. The background for the changes might be increases in soil pH, alterations in botanical composition of leys and less input of minerals from fertilizers. In order to evaluate the plant and animal trace element status and suggest actions for improvements, a farm survey on Norwegian organic sheep and dairy farms was undertaken.

## Methods

Seven organic farms within each of the two regions Coast (1) and Mountain (2), having a high density of sheep farms, and East (3) and Middle (4), having a high density of dairy farms were selected (Fig. 1). All farms had maintained organic plant production for more than three years and organic animal production for at least one year. On each farm, three leys were investigated. Soil was sampled (0-20 cm) after the first harvest in 2001 and herbage was sampled at each cut in 2001 and 2002. Five animal blood samples taken late in the indoor season (April-May) in 2002 were pooled from each farm. DTPA was used to extract soil Fe, Cu, Mn and Zn. Mo was extracted with oxalic acid, Co with acetic acid and Se with $KH_2PO_4$. Zinc, Fe, Mn, Cu, Mo and Co were analysed by ICP-AES and Se by ICP-MS.

Official methods according to the Association of Analytical Chemists were used for the determination of total herbage Zn, Fe, Mn, Cu, Mo and Co concentrations by dry ashing. Zinc, Fe, Mn, Cu, Mo and Co were determined by ICP-AES. For Se determination, herbage samples were digested in nitric and perchloric acids on a heating block. Selenium was reduced with HCl, and diluted with water and determined with HG-AAS.

Animal blood plasma Cu concentration was determined by flame AAS and plasma vitamin $B_{12}$ concentration as an indicator of the Co status was determined with the Dualcount Solid Phase No Boil radioassay kit. Whole blood Se concentration was determined by sodium borhydride reduction and AAS. Plasma vitamin E concentration was determined as α- and γ-tocopherol concentration by HPLC. Details of the method are presented in Govasmark et al., 2005a, Govasmark et al., 2005b, Govasmark et al., 2005c.

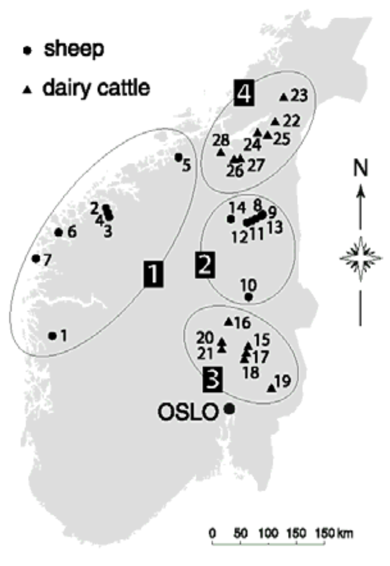


Fig. 1. Map of southern Norway indicating the location of 27 farms in the Coast (1), Mountain (2), East (3), and Middle (4) regions.

**Results and discussion**

The soil from the Middle region (Fig. 1) had the highest concentration of extractable Cu but lowest content of Zn, the East region had the highest soil Mo concentration and the Coast region had the lowest Mn and Co soil extractable concentration. No differences in soil extractable Fe was found between the regions. Except for Zn, there were no significant relationships between soil and plant concentration of the different minerals. These results indicate that the extracting procedures used did not draw out the plant available pool of soil Fe, Mn, Cu, Mo and Se.

Soil physical measurements did not explain much of the variation in the soil extractable trace element concentration, whereas the soil texture did explain some. Since only extractable soil Zn was found to be correlated with herbage concentration, the relationship between soil and herbage trace element concentrations did not provide precise information for predicting the herbage trace element concentration.

The median herbage trace element concentrations of all observations in the 1$^{st}$ and 2$^{nd}$ cut are presented in table 1. The herbage trace element concentration was generally higher in the second cut. The herbage Mo concentration was highest in the East region whereas no regional differences in Cu, Fe and Co concentrations were found. The lowest herbage Zn and Mn concentrations were found in the Middle region. The highest herbage Se concentration was found in the Coast and Middle region, most probably because of aerial sea deposition. The herbage trace element concentrations, except for Zn, were all above the suggested critical plant growth limiting concentrations, and were expected not to limit plant growth (Table 1).

Table 1. The median (10$^{th}$ – 90$^{th}$ percentile) herbage trace element concentrations for 28 farms in two years and the critical concentration (mg kg$^{-1}$) for plant growth.

| | Herbage concentration (mg kg$^{-1}$ DM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Zn | Fe | Mn | Cu | Mo | Co | Se |
| 1st cut | 19<br>(14-34) | 50<br>(36-88) | 34<br>(22-86) | 5.3<br>(3.9-6.8) | 1.5<br>(0.6-4.8) | <0.05<br>(<0.05-0.08) | <0.01<br>(<0.01-0.03) |
| 2nd cut | 21<br>(16-37) | 84<br>(52-171) | 66<br>(36-205) | 7.0<br>(5.7-9.3) | 3.3<br>(1.6-10.1) | 0.06<br>(<0.05-0.15) | 0.02<br>(<0.01-0.06) |
| Critical conc. | 20 | 50 | 20 | 4 | 0.1 | | |

In the present study the Cu, Zn, Mo and Co concentrations in red clover were higher than in grass herbage. A mixed model statistical analysis showed that the presence of clover increased the herbage concentration of Cu, but it decreased the Mn concentration, while forbs increased the herbage Zn and Cu concentrations. Red clover is reported to have a lower Cu:Mo ratio than grasses (Frøslie & Norheim, 1983), and herbage diets with high red clover content have a potential for inducing secondary Cu deficiency in animals. Contrary results were obtained in the present investigation, because the Cu:Mo ratio was lower in the herbage than in red clover. The statistical analysis also revealed that the herbage Cu concentration increased with increasing clover proportion whereas the herbage Mo concentration was unaffected.

The herbage Cu:Mo ratio, though variable, was generally low, 76 % below 6, which is suggested as the lower Cu:Mo ratio to avoid secondary Cu deficiency. The herbage Cu:Mo ratio decreased with increasing herbage Mo concentration (Fig. 2). The Cu:Mo ratio is important because Mo can exert a depressive effect on Cu availability and absorption in animals but the interrelation only exerts on Cu in the presence of sulphate. These interrelationships will only occur when the herbage levels of S are at least 4 g kg$^{-1}$ DM combined with herbage Mo concentration above 3 mg kg$^{-1}$ DM (Fisher, 2004). Ninety-six percent of all herbage samples in the first and 67 % in the second cut contained less than 2 g S kg$^{-1}$ DM herbage in the present investigation. The maximum herbage S concentration was 3.10 g kg$^{-1}$ DM, indicating that Mo induced Cu deficiency presumably is not a problem. According to the dietary needs of ruminants, primary Zn, Co and Se deficiency can be expected because of low herbage concentrations.

The blood plasma Cu and $B_{12}$ (except one sheep herd) concentration were within the suggested normal range set by the Norwegian Veterinary Institute. The median (10$^{th}$, 90$^{th}$ percentile) whole blood Se concentrations were 0.10 (0.04, 0.15) µg g$^{-1}$ in dairy cattle and 0.14 (0.03, 0.26) µg g$^{-1}$ in sheep. Vitamin E concentrations were 4.2 (2.7, 8.4) mg L$^{-1}$ in dairy cattle and 1.3 (0.9, 2.4) mg L$^{-1}$ in sheep.

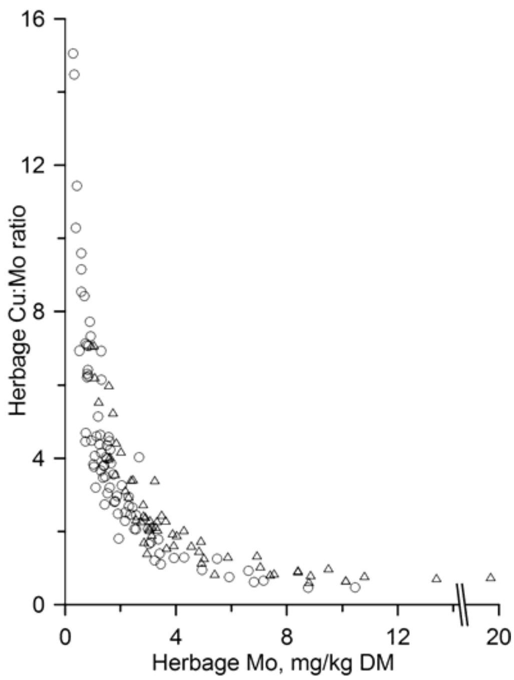


Fig. 2. Cu:Mo ratio in herbage from 1$^{st}$ and 2$^{nd}$ cuts on 27 farms in two years according to herbage Mo-concentration.

Results from the present investigation show that despite the low herbage Cu:Mo ratio and Co concentrations, the blood plasma Cu and Co ($B_{12}$) concentrations were within the suggested standards for blood plasma Cu and Co concentration. There were no higher animal blood Cu or Co concentrations on farms where trace element enriched supplements were used. This demonstrated that the low Cu:Mo relationship did not induce Cu deficiency and that the herbage Co concentration was sufficient to meet the animal dietary need. If the supplement of S increases in organic farming, this might induce Cu deficiency, requiring increased feed Cu concentration. In contrast, the Se feeding practise was generally insufficient to meet the needs of dairy cattle in the East region, but it was found to be sufficient for sheep. The six farms having the lowest animal blood Se concentration in the present study did not use Se enriched feed supplements. The vitamin E fed through the herbage and supplement was sufficient to meet the dietary need of the dairy cattle herds but insufficient for the sheep herds. Hay was the most common feed for sheep, whereas silage was mostly used for dairy cattle.

Based on these observations it was concluded that today's feeding practice for sheep and dairy cattle herds on organic farms was sufficient regarding Cu and Co, but Se should be supplemented to all animals and vitamin E should be supplemented to animal diets consisting mainly of hay.

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# Essential trace elements in human nutrition

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**Minerals and essential trace elements in Nordic Nutrition Recommendations 2004**

| | | I[1] | II[2] | III[3] | IV[4] |
|---|---|---|---|---|---|
| Ca | mg | 800 | 100 | 400 | 2 500 |
| P | mg | 600 | 80 | 300 | 4 000 |
| Mg | mg | 320 | 35 | | |
| Na | g | 2.5 | | | |
| K | g | 3.3 | 0.35 | 1.6 | |
| Fe | mg | 9 – 15 | 1.6 | 6 | 25 |
| Zn | mg | 8 | 1.1 | 4.5 | 25 |
| I | µg | 150 | 17 | 70 | 600 |
| Se | µg | 45 | 4 | 20 | 300 |
| Cu | mg | 0.9 | 0.1 | 0.4 | 5 |
| Mn | mg | (2) | | | |
| Mo | µg | (45) | | | |
| F | mg | (3.5) | | | |

1) Recommended intake (RI) Mean values for adult males and females
2) Recommended nutrient density (content per MJ) for planning diets for groups of individuals
3) Estimated lower level of intake (LI)
4) Estimated upper level (UL) for average daily intake

**Major sources of minerals and trace elements (%) (Riksmaten Sweden 1997-98).**

| | Ca | P | Fe | Mg | Na | K | Zn | Se |
|---|---|---|---|---|---|---|---|---|
| Dairy products | 62 | 25 | 2 | 17 | 8 | 17 | 23 | 17 |
| Cereals | 13 | 24 | 31 | 25 | 27 | 14 | 23 | 12 |
| Fruit & vegetables | 9 | 11 | 20 | 24 | 14 | 38 | 10 | 6 |
| Meat prod | 3 | 17 | 30 | 9 | 33 | 13 | 7 | 28 |
| Fish | 2 | 4 | 4 | 3 | 8 | 3 | 3 | 23 |

**Special public health aspects**

Interactions, bioavailability concerns:

- Fe,
- Zn
- Ca,
- Mg

**Special clinical conditions**

Ca – osteoporosis
Fe – iron deficiency anemia
I – iodine deficiency (goiter)
Magnesium – 'hard water ' – cardiovascular disease
(Selenium – cardiovascular disease?)

**Means to increase the intake**

Dietary guidelines

- Milk – Ca
- Meat – Fe
- Fruit and vegetables
- Cereals

Fortification

- iodine,
- selenium,
- (iron)

Supplementation

- iron;
- multi mineral products

# Selenium supplemented fertilization - effects on the selenium content of foods and the selenium intake in Finland

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## Introduction

In the 1970s results of the several studies indicated that in Finland the availability of selenium (Se) to plants was low due to the climatic and geochemical reasons. The Se content of domestic animal feeds and foods was very low and attention was given to the possible health effects of the low average Se intake of the population, only about 0.02-0.03 mg per day (Koivistoinen, 1980, Mutanen, 1984). In 1984 an official decision was made by the Ministry of Agriculture and Forestry to supplement compound fertilizers with sodium selenate to improve the quality of Finnish foods. The objective was to guarantee the adequate Se contents in agricultural products and to increase the average Se intake of the population to the recommended level. Since the beginning of the Se supplementation the Se levels in soils, fertilizers, feeds, basic foods, human serum and the average Se intake has been monitored. During this 20-year period the Se supplementation level has been revised twice. First two supplementation levels were used: 6 mg/kg for fertilizers intented for hay and fodder production and 16 mg/kg for fertilizers intented for cereal production. In 1990 uniform 6 mg/kg level was applied for all fertilizers and in 1998 the supplementation level was raised to10 mg/kg.

Se content of 15 basic foods were monitored regularly first in University of Helsinki (1984-1996), then in the Agrifood Research Finland (1998-) (cereals, wheat and rye flour and bread, milk, cheese, eggs, fish, potato, white cabbage) and in the National Veterinary and Food Research Institute (meat and liver). Organically grown foods were studied during 2001-2002. Occasionally some other food items were analyzed.

## Results

The effect of Se fertilization was distinct. In the growing season 1985 the average Se content of spring cereals increased over 20-fold, from 0.01 to 0.23 mg $kg^{-1}$ dw and remained at the level of 0.20-0.30 mg $kg^{-1}$ dw during the years when supplementation level was 16 mg $kg^{-1}$ fertilizer (Ekholm, 1997). Winter cereals were not affected as much as spring cereals due to the different cultivation and fertilization practice. Only Se supplementation of nitrogen fertilizer in 1996 has raised the Se content of winter cereals to about 0.1 mg $kg^{-1}$ dw. In 2004 (supplementation level 10 mg $kg^{-1}$ fertilizer) the average Se content of domestic cereals were approximately 0.1 mg $kg^{-1}$ dw which was the original target value (Table I). However the variation between the farms was large <0.01-0.30 mg $kg^{-1}$ dw.

Selenium contents of flours and breads (Table 1) have increased 10-20-fold. However, Se contents of flours and bread does not necessarily correlate with the Se content of domestic grain, but is also affected by the proportion of imported grain in the milling. During the years of crop failure the amount of imported grain can be as high as 100%. Recently most of the imported grain has been of European origin, where the Se content is often lower than in Finnish grain. Thus the high proportion of imported grain lowers the Se content of flours and breads (Eurola et al., 2003). In organic cultivation Se content of cereals was low, about 0.01-0.02 mg $kg^{-1}$ dw. At the moment it is not permitted to add Se to organic fertilizers and the Se supplementation does not reach organically grown plants.

Table 1. Se content of wheat and rye grains in Finland in 1984 and 1998-2004.

| Year | Selenium content mg kg$^{-1}$ dw. | | | | | |
|---|---|---|---|---|---|---|
| | n | Spring wheat | n | Winter wheat | n | Rye |
| Silo samples from mills | | | | | | |
| 1984[a] | 12 | 0.012 ± 0.007 | | | 10 | 0.009 ± 0.003 |
| 1998 | 3 | 0.076 ± 0.011 | 3 | 0.052 ± 0.010 | 2 | 0.066 ± 0.000 |
| 1999 | 4 | 0.130 ± 0.010 | 2 | 0.097 ± 0.025 | 1 | 0.120 |
| 2000 | 3 | 0.160 ± 0.014 | 2 | 0.130 ± 0.008 | 2 | 0.110 ± 0.006 |
| 2001 | 4 | 0.160 ± 0.050 | 3 | 0.091 ± 0.022 | 3 | 0.130 ± 0.027 |
| 2002 | 3 | 0.180 ± 0.034 | 3 | 0.100 ± 0.010 | 2 | 0.070 ± 0.023 |
| 2003 | 3 | 0.120 ± 0.008 | 3 | 0.085 ± 0.007 | 2 | 0.075 ± 0.005 |
| 2004 | 3 | 0.140 ± 0.035 | 2 | 0.076 ± 0.051 | 2 | 0.092 ± 0.029 |
| Farm samples | | | | | | |
| 1984 | | | | | | |
| 1999 | 13 | 0.150 ± 0.021 | 13 | 0.120 ± 0.015 | 22 | 0.130 ± 0.083 |
| 2000 | 14 | 0.190 ± 0.082 | 14 | 0.110 ± 0.042 | 12 | 0.110 ± 0.048 |
| 2001 | 21 | 0.130 ± 0.080 | 14 | 0.140 ± 0.051 | 15 | 0.084 ± 0.063 |
| 2002 | 44 | 0.150 ± 0.075 | 15 | 0.130 ± 0.059 | 20 | 0.072 ± 0.057 |
| 2003 | 32 | 0.140 ± 0.070 | 21 | 0.058 ± 0.032 | 27 | 0.079 ± 0.042 |

[a] Ministry of Agriculture and Forestry 1994

Milk was the first foodstuff indicating the effect of Se supplementation. Se content of milk doubled immediately when the outdoor feeding season begun and reached later average level of 0.2 mg kg-1 dw. The Se content of milk varies according to the season, being highest in the indoor feeding season and beginning to decrease in the outdoor feeding season. Present Se contents of milk, cheese and other basic foods are presented in table 2.

The Se intake meets well the international and national recommendations in Finland. The estimated average daily Se intake was slightly under 0.070 mg/day /10 MJ in 2004. It satisfies RDA and DRI daily Se intake recommendations of 0.055 mg. The most important Se sources are milk and other dairy products, meat and meat products. Together they account for nearly 70% of the total Se intake.

In Finland the supplementation of fertilizers with Se has proved to be an effective and safe way to improve the Se intake nationwide. Uniform geochemical conditions make the system relatively controlled. In this method plants take up selenate and convert it to organic Se compounds, mainly selenomethionine. This increases the Se content of foods/feeds of both plant and animal origin and have positive effect on human and animal Se intake.

Table 2. Se content of basic foods in Finland in 1975/77 and 2004.

| Food | Se content mg $kg^{-1}$ dw. | | | |
|---|---|---|---|---|
| | n | Mean 1975/77[a] | n | Mean 2004 |
| Milk, standardized 3.5% fat | 19 | 0.02 | 16 | 0.220 |
| Milk, standardized 1.5% fat | - | - | 16 | 0.180 |
| Cheese, Edam-type | 5 | 0.07 | 16 | 0.330 |
| Eggs | 4 | 0.41 | 16 | 1.010 |
| Rye bread | 7 | 0.02 | 30 | 0.058 |
| Rye flour | 10 | 0.01 | 6 | 0.054 |
| Wheat bread | 5 | 0.01 | 17 | 0.099 |
| Wheat flour | 9 | 0.02 | 10 | 0.110 |
| Potato | 20 | <0.01 | 4 | 0.033 |
| White cabbage | 5 | <0.01 | 4 | 0.160 |
| Bovine meat | 32 | 0.04 | 10 | 0.340 |
| Bovine liver | 8 | 0.24 | 25 | 0.950 |
| Pig meat | 4 | 0.20 | 16 | 0.460 |
| Pig liver | 4 | 1.38 | 29 | 1.110 |
| Rainbow trout, farmed | 6 | 0.76 | 4 | 0.750 |
| Baltic herring | 5 | 0.78 | 12 | 0.740 |

[a] Koivistoinen, 1980

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# The role of manganese application on quality of potato tubers

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## Introduction

Nutrient management is a critical component for successful crop production. The potato plant is no exception in its nutrient requirements. Maintaining adequate levels of all essential nutrients is important because healthy plants have a greater disease tolerance and cope much better with adverse growing conditions that can significantly reduce yield and quality.

Manganese (Mn), required by plants in small quantities is one of the micronutrients that are just as important as the macronutrients that are required in relatively large amount. Growers should carefully follow recommendations for micronutrients to avoid unnecessary costs and possible toxic effects or deleterious interactions with other nutrients. Although potatoes do require small amounts of various micronutrients and soils used for potato production are rarely deficient in these (Gaujers, 1980), at the same time micronutrient deficiencies have been reported for potatoes in many potato growing regions.

The aim of this work was to verify theses if adding of special micronutrients (manganese chelate) improves common scab management in Vidzeme Region in Latvia.

## Materials and methods

Studies were carried out at Priekuli Plant Breeding Station under Vidzeme (North-east part of Latvia) conditions to examine the effects of foliar applied Mn on the productivity and quality of seed potatoes. Cultivar Zile (middle late maturing table cultivar) were used in these studies, as it is the leading in range of unresistant to common scab in National variety list in Latvia.

Because the main aim was to exam effect of Mn chelate on management of common scab there were investigations in three different backgrounds of fertilizations based: one-normal (recommended in region) and two provocations phone (facilitating common scab infection)

- with incorporating of fresh manure in spring;
- with incorporating of liming material in spring.

Trials were conducted on soddy podzolic light loam soil ($pH_{HCl}$ –5.8) with no previous history of micronutrient use. The foliar treatment was applied at the time of early tuber bulking. The sources of the foliar micronutrient applications included commercial formulations of manganese chelate (Mn EDTA 4.8%, 6 l/400l $H_2O$).

The test plots were managed using standard cultural practices for seed potato. Harvested tubers were graded into seed and consumption market classes based on tuber diameter. Seed grade included tubers between 25 and 80 mm diameter. Infection with common scab was determined according to methodic recommended by State Plant Protection Service.

## Results and discussion

Data shows that foliar-applied Mn practically affected no tubers yield level. Small influence was found on fertilizing background with stable manure using (Figure 1). In average 9.5% highest amount of tuber for consumption in all fertilizations backgrounds under application of Mn chelate were found.

Effects of manganese chelate treatments on common scab are shown in Figure 2. The data certified positive effect of treatment to manage common scab: manganese chelate reduced common scab. In accordance with many recommendations to minimize the risk of infection by common scab soil $pH_{HCl}$ should be kept between 5.8 and 6.4 (Ruza, 2001, Walworth, 1992). Results on infection with common scab acquired in background with incorporating of liming material in spring certify this accordingly affirming information given by other authors (Swiader, 1995). Acquired data confirm the role of Mn in the maintaining of good quality of potato yield, especially when elevated soil pH is high.

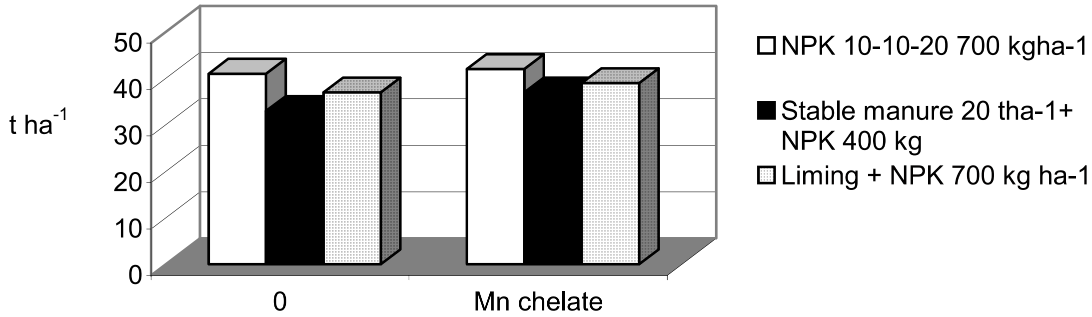


Fig. 1 The influence of Mn chelate on potato Zile yield.

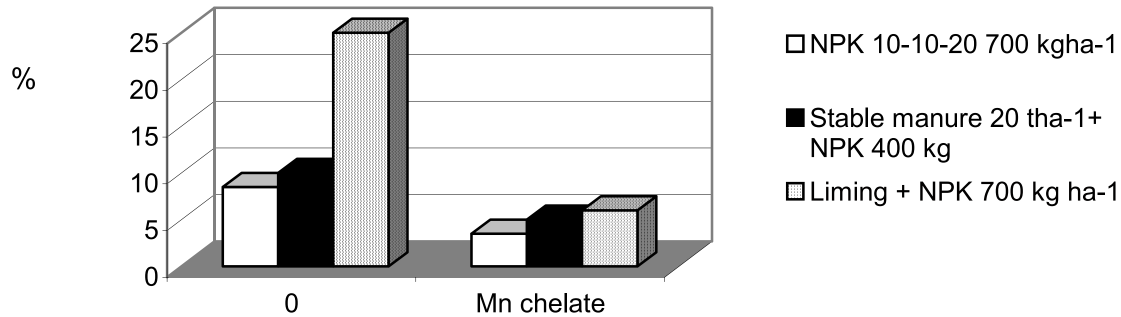


Fig 2. The influence of Mn chelate on common scab in potato Zile.

The effects of Mn chelate treatment were not found on starch content of potato (Figure 3).

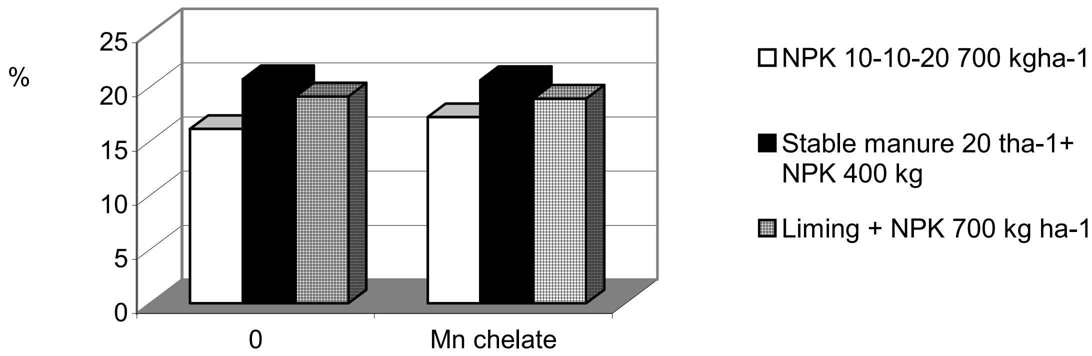


Fig.3. The influence of Mn chelate on potato starch content.

**Conclusions**

Successful potato production depends on numerous factors that can be controlled by the grower. Soil pH must be carefully controlled to avoid loss of tuber quality through common scab *Streptomyces scabes)* infection. Foliar application of commercial formulations of manganese chelate (Mn EDTA 4.8%, 6 l/400l $H_2O$) improves management with common scab.

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Walworth J. 1992. Field Crop Recommendations for Alaska. Crop Production and Soil Management Series No. FGV-00643. 11.

# Trace element content of rye (*Secale cereale* L.) cultivars in official variety trials in Finland during 1998-2002

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**Introduction**

In Finland over 90% of rye is used as food, the consumption being about 90 milj. kg annually (Tike, 2004). Domestic production of rye is not sufficient and variable amount is imported depending on the annual production area, yield and quality. Rye is mainly consumed as full grain bread products containing significant amounts of dietary fiber and other nutritionally important components, like mineral and trace elements, vitamins and fenolic compounds. Sour dough baking process also reduces the amount of fytic acid, which may have beneficial effects on the availability of the mineral and trace elements. The Ministry of Agriculture and Forestry started in 1997 a national rye program to improve competitiveness of rye in the market (production, quality and processing). The present study focuses on the nutritional composition of different rye cultivars. Cadmium was studied as a toxic risk factor.

**Methods**

Rye samples were collected during 1998-2002 from the official variety trials at 9 locations in Finland. Originally the material consisted 300 samples of 32 rye cultivars or lines from which the cultivars Parviainen (landrace), Esprit, Picasso, Kaskelott (hybrid ryes), Riihi, Voima, Akusti, Elvi, Amilo and Walet (population cultivars) were selected for statistical analyses (186 samples). The rye grains were sorted by a 1.5 mm sieve and milled by Falling Number hammer mill using 1.0 mm sieve. Trace elements were analysed after wet digestion by ICP (Cu, Zn, Mn, Fe), ICP-MS (Cd) or grafite furnace atomic absorption spectrometry (Se). All the methods were accredited.

**Results**

The essential trace element contents were in general negatively related to the yield level: the higher the yield, the lower the trace element content tended to be. Only the relationship between Se and yield was positive and Mn content was not dependent on the yield level. Thus, except for Mn the cultivar comparisons were based on linear mixed models in which the relation between each trace element and yield was described by a straight line. The levels of the lines and for Cu and log-transformed Cd also the slopes of the lines varied between the cultivars.

The rye cultivars were compared at the constant yield level of 4000 kg $ha^{-1}$ (Table I). The cultivars could be divided into the following three groups: the landrace Parviainen and the population cultivars Riihi and Voima had the highest mean trace element contents, the hybrid ryes Esprit, Kaskelott and Picasso the lowest contents and the other cultivars were between these two groups. The hybrid ryes have generally large grains with higher starch content and in normal conditions bigger yields than population or landrace cultivars. Grain size was not related to trace element contents after allowing for yield level.

The trace element contents of rye were generally at the same level as in the previous studies. The mean Se content of rye increased 3-fold by the end of the 5-year study period due to the increment in the Se supplementation level of the fertilizers in 1998. The mean Cd content of rye was low. In all the samples the Cd concentrations were below the maximum Cd level of 0.1 mg $kg^{-1}$ fresh weight set by EU.

Table I. Estimated means of copper, zinc, manganese, iron, selenium and cadmium for different rye cultivars in the official variety trials in Finland during 1998-2002 at the yield level of 4000 kg $ha^{-1}$. (dw = dry weight, n = number of samples, CI = confidence interval).

| Cultivar | n | Trace element content mg $kg^{-1}$ dw | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | Zn | Mn | Fe | Se | Cd |
| **Hybrid ryes** | | | | | | | |
| Picasso | 22 | 4.0 | 33 | 23 | 40 | 0.053 | 0.030 |
| Kaskelott | 12 | 4.5 | 34 | 25 | 41 | 0.054 | 0.032 |
| Esprit | 13 | 4.5 | 32 | 23 | 42 | 0.056 | 0.022 |
| **Population cultivars** | | | | | | | |
| Walet | 14 | 4.8 | 36 | 26 | 48 | 0.056 | 0.027 |
| Elvi | 31 | 5.1 | 36 | 27 | 46 | 0.061 | 0.030 |
| Amilo | 32 | 5.0 | 35 | 27 | 47 | 0.058 | 0.027 |
| Akusti | 9 | 4.6 | 34 | 29 | 51 | 0.065 | 0.020 |
| Voima | 14 | 5.3 | 37 | 30 | 52 | 0.068 | 0.026 |
| Riihi | 30 | 5.6 | 38 | 29 | 57 | 0.075 | 0.025 |
| **Landraces** | | | | | | | |
| Parviainen | 9 | 5.7 | 37 | 32 | 55 | 0.093 | 0.028 |
| **All cultivars** | | | | | | | |
| Mean | | 5.0 | 35 | 27 | 47 | 0.064 | 0.027 |
| 95% CI for the mean | | 4.4-5.5 | 31-39 | 24-30 | 40-57 | 0.043-0.085 | 0.018-0.041 |

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# Contents of some essential trace elements in grass from cultivated meadows and vegetation from natural pastures in Western Norway: Is there an adequate supply to sheep?

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**Keywords**

Essential trace elements, grass cultivated meadows, natural pastures, sheep

**Introduction**

Several investigations have indicated low concentrations of micronutrients in soil and plants in Norway, and farmers have reported trace elements deficiencies in sheep. Sheep`s average daily need of micronutrients is as follows: Mn, >30 mg/kg DM; Fe, 100-500 mg/kg DM; Co, >0.11 mg/kg DM; Cu, 5-15 mg/kg DM; Zn; >30 mg/kg DM. The aim of the present study was to see if sheep grazing cultivated meadows and natural pastures in some areas of Western Norway receive an adequate supply of these trace elements. Knowing that lambs in this part of the country usually graze on natural pastures from the age of 5-7 weeks, we found it particularly interesting to investigate the vegetation from natural pastures.

**Materials and methods**

46 samples of grass were taken from cultivated meadows between the end of May and the middle of October 2001 from 11 sheep farms in the western regions of Sogn and Fjordane county. Areas where farmers or advisers suspected lack of trace elements were selected. 33 samples from 12 species of vegetation from natural pastures were taken during the period July - September 2001 from the same regions. The name of this project was “Coastlamb” and the results are listed by this name in the tables below.

In addition 22 samples of 8 current species from Solund, Ytre Sogn, were taken in late August 2002, and in late June 2003 and 2004. The results from this sampling are identified as “Solund” in the tables below.

The present results are compared with data from other Norwegian investigations of micronutrients in the same plant species.

**Results**

Table 1 show that the concentration of Co and Zn are low in grass from cultivated meadows. Most of the samples have a normal Cu-content, but 28% of them show a Cu/Mo ratio lower than the optimal limit.

Table 1. Contents of Mn, Fe, Co, Cu, Zn, and Mo in 46 samples of grass from cultivated meadows in Western Norway.

| Micronutrient | Mn | Fe | Co | Cu | Zn | Mo |
|---|---|---|---|---|---|---|
| Mg/kg DM | 142 | 111 | 0.12 | 7.27 | 29.7 | 1.16 |
| Samples with low values (%) | 11 | 67 | 57 | 13 | 56 | 13 |

Table 2 . Contents of Mn, Fe, Co, Cu, Zn, and Mo in 9 plant species from natural pastures in Norway (mg/kg DM). The results from “Coastlamb” and ”Solund” are in bold.

| | Location | | Mn | Fe | Co | Cu | Zn | Mo |
|---|---|---|---|---|---|---|---|---|
| **A. Calluna vulgaris (Heather)** | | | | | | | | |
| **Coastlamb** | **Ytre Sogn** | | **355** | **64** | **0.087** | **7.8** | **18** | **0.17** |
| **Solund** | **Solund** | | **831** | **63** | **0.054** | **6.9** | **19** | **0.19** |
| Gjengedal 1992 | Sogndal | | 346 | | | 8.6 | 17 | |
| | Risdalsheia (Aust-A) | | 404 | | | 9.2 | 27 | |
| Berthelsen et al. 1995 | Sørlandet | | | | | 6.9 | 26 | |
| | Trøndelag | | | | | 5.3 | 14 | |
| Kålås 2003 | Nordmøre | | | | | 5.6 | 16 | |
| Brekken & Steinnes 2004 | Lund, Rogaland | | | | | | 27 | |
| **B. Molinia coerulea (Purple moorgrass)** | | | | | | | | |
| **Coastlamb** | **Ytre Sogn** | | **85** | **72** | **0. 01** | **9.7** | **33** | **0.27** |
| **Solund** | **Solund** | | | | **0.017** | **5.3** | **31.5** | **0.14** |
| Garmo et al. 1986 | Lom/Vågå/Folldal | | 143 | 106 | | 5.3 | 38 | 0.27 |
| Garmo & Nedkvitne 1992 | Eksingedalen | | | | | 14.6 | | 0.36 |
| Gjengedal 1992 | Sogndal | | 97 | | | 8.7 | 46 | |
| | Risdalsheia (Aust-A) | | 105 | | | 8.5 | 66 | |
| Hamar 2000 | Vest-Agder | | 72 | 104 | 0.08 | 6.8 | 44 | 0.5 |
| Brekken & Steinnes 2004 | Lund, Rogaland | | | | | | 52 | |
| **C. Deschampsia flexuosa (Wavy hairgrass)** | | | | | | | | |
| **Coastlamb** | **Ytre Sogn** | | **176** | **55** | **0.115** | **5,1** | **21** | **0.47** |
| Garmo et al. 1986 | Lom/Vågå/Folldal | | 278 | 50 | | 3.5 | 26 | 0.21 |
| Garmo & Nedkvitne 1992 | Eksingedalen | | | | | 13.5 | | 0.71 |
| Gjengedal 1992 | Sogndal | | 169 | | | 5.5 | 36 | |
| Hamar 2000 | Vest-Agder | | 162 | 48 | 0.07 | 3.7 | 28 | 0.28 |
| Brekken & Steinnes 2004 | Lund, Rogaland | | | | | | 33 | |
| **D. Betula pubescens (Downy birch)** | | | | | | | | |
| **Coastlamb** | **Ytre Sogn** | **leaf/twig** | **203** | **45** | **0.332** | **5.0** | **149** | **0.02** |
| **Solund** | **Solund** | **leaf** | | | **0.15** | **5.3** | **102** | |
| Garmo et al. 1986 | Lom/Vågå/Folldal | leaf | 489 | 186 | | 6.1 | 181 | 0.1 |
| Garmo & Nedkvitne 1992 | Eksingedalen | leaf | | | | 19.1 | | 0.11 |
| Gjengedal 1992 | Sogndal | leaf | 380 | | | 8.0 | 233 | |
| | | twig | 99 | | | 7.3 | 210 | |
| | Risdalsheia (Aust-A) | leaf | 362 | | | 6.1 | 352 | |
| | | | 126 | | | 6.7 | 339 | |
| Berthelsen et al. 1995 | Sørlandet | leaf | | | | 4.9 | 267 | |
| | | twig | | | | 5.2 | 282 | |
| | Trøndelag | leaf | | | | 4.5 | 85 | |
| | | twig | | | | 4.8 | 128 | |
| Garmo 1999 | Fjellbeite sørnoreg | leaf | 489 | 186 | | 6.1 | 181 | 0.1 |
| Hamar 2000 | Vest-Agder | | 162 | 48 | 0.07 | 3.7 | 28 | 0.28 |
| Reimann et al. 2001 | Berlevåg | leaf | 1470 | 82 | 0.044 | 5.7 | 205 | 0.03 |
| Kålås 2003 | Nordmøre | leaf | | | | 3.2 | 129 | |
| | | shoot | | | | 4.8 | 114 | |
| Halse et al. 2003 | Førde | leaf | 282 | 61 | | 6.9 | 163 | |
| Brekken & Steinnes 2004 | Lund, Rogaland | leaf | | | | | 316 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **E. Vaccinium myrtillus (Bilberry)** | | | | | | | | |
| **Coastlamb** | **Ytre Sogn** | **leaf/ twig** | **742** | **46** | **0.051** | **6.8** | **18** | **0.06** |
| Garmo et al. 1986 | Lom/Vågå/Folldal | leaf | 529 | 61 | | 6.2 | 36 | 0.09 |
| Garmo & Nedkvitne 1992 | Eksingedalen | leaf | | | | 18.6 | | 0.12 |
| Gjengedal 1992 | Sogndal | leaf | 900 | | | 11.0 | 24 | |
| | | twig | 1100 | | | 8.3 | 58 | |
| | Risdalsheia (Aust-A) | leaf | 1200 | | | 9.2 | 23 | |
| | | twig | 1090 | | | 9.3 | 61 | |
| Hamar 2000 | Vest-Agder | leaf/ twig | 618 | 46 | 0.05 | 6.4 | 30 | 0.12 |
| Reimann et al. 2001 | Berlevåg | leaf | 1900 | 45 | 0.36 | 6.5 | 14 | 0.03 |
| Kålås 2003 | Nordmøre | leaf | | | | 5.0 | 13 | |
| | | shoot | | | | 6.1 | 27 | |
| Brekken & Steinnes 2004 | Lund, Rogaland | leaf | | | | | 16 | |
| | | twig | | | | | 55 | |
| **F. Salix spp (Osier/Great sallow)** | | | | | | | | |
| **Coastlamb** | **Ytre Sogn/Sunnfjord** | **leaf, Osier** | **290** | **70** | **1.56** | **5.9** | **138** | **0.21** |
| **Solund** | **Solund** | **leaf, Great sallow** | | | **0.12** | **7.9** | **78** | |
| Garmo & Nedkvitne 1992 | Eksingedalen | | | | | 14.7 | | 0.24 |
| Garmo 1999 | Fjellbeite sørnoreg | leaf, Osier | 1084 | 90 | | 7.1 | 166 | 0.13 |
| Halse et al. 2003 | Førde | leaf, Great s. | 46 | 47 | | 9 | 183 | |
| **G. Potentilla erecta (Tormentil)** | | | | | | | | |
| **Solund** | **Solund** | | **416** | **163** | **0.205** | **8.48** | **49.7** | **0.56** |
| **H. Juniperus communis (Juniper)** | | | | | | | | |
| **Solund** | **Solund** | **needles** | **150** | **33** | **0.202** | **4.38** | **28.5** | **0.06** |
| **I. Festuca rubra (Red fescue)** | | | | | | | | |
| **Coastlamb** | **Ytre Sogn/Sunnfjord** | | **176** | **39.6** | **0.177** | **3.73** | **27.5** | **0.455** |

*Calluna vulgaris* (heather) and *Molinia coerulea* (purple moorgrass) are two of the main species in natural pastures on the western coast of Norway. The other species shown above are also important plants for sheep grazing natural pastures.

The concentrations of Co, Zn and Cu differed a lot between species. Table 2 shows that the most important sources of Co in western Norway are *Salix spp* (osier), *Potentilla erecta* (tormentil), *Juniperus communis* (juniper), and *Festuca rubra* (red fescue).

The dominant Zn-source in our material is *Betula pubescens* (downy birch), but also *Salix spp* (osier) has a high concentration level.

The Cu/Mo ratio is very high in many of the species from natural pastures, particularly in juniper and bilberry, but also in birch.

## Conclusion

Sheep and lambs grazing or being fed from grass-dominated meadows in the present study area do not get an adequate supply of Co, Zn and in some instances Cu, and therefore need extra supply of these nutrients. On the other hand sheep and lambs grazing natural pastures with a wide range of vegetation available seem to get an adequate supply of micronutrients.

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# Green fodder crops and dicotyledonous weeds as sources for micronutrients in ruminant diets

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**Summary**
It has been investigated whether green fodder crops and dicotyledonous weeds may contribute to more balanced diets for ruminants, according to Co, Cu and Mo, than perennial grasses do. *Vicia faba* was found useful for diets otherwise low in Co or with a high Cu:Mo-ratio. To increase the Cu:Mo-ratio *Lolium multiflorum* and *Raphanus sativus* were the most appropriate species.

Keywords: Cobalt, copper, molybdenum, forages

**Introduction**
Ingestion of diets deficient, imbalanced or excessively high in cobalt (Co) copper (Cu) and/or molybdenum (Mo) may induce deprived feed utilisation, production and fertility in ruminants. In coastal areas of Norway, a low level of cobalt and a low Cu:Mo ratio is often found in the soil and roughage, and copper and cobalt deficiency occasionally occurs in cattle and sheep herds in these areas. On the other hand, a excessively high Cu:Mo ratio is found in certain inland areas of Norway. To investigate whether other forages than perennial grasses may contribute to a more balanced diet for ruminants, two studies have been conducted.

**Material and methods**
In year 2000 seven green fodder species were grown in six field experiments (Study A). All were located near the coast (60ºN - 64ºN) at farms where deficits or imbalance in the supply of Co, Cu and/or Mo were expected. Samples from each fodder species, and a representative sample from the main source for roughage at the farm (perennial meadows), were collected for analyses of Co, Cu and Mo. Soil samples were also collected and analysed. In the spring 2001, samples of four dicotyledonous weeds and two perennial grass species were collected from perennial meadows at four different fields (Study B). The same chemical analyses as in Study A were conducted.

**Results and discussion**
The low Co-content found in grass and meadow samples (Table 1) corresponds well with other investigations in Norway (Synnes and Øpstad, 1995). The variation between sites was significant. Only *Vicia sativa* and *Hordeum vulgare* were found to be significantly different amongst the species. Although the mean Co-contents in *Vicia sativa* exceeded the recommended level of 0.1 mg $kg^{-1}$ dry matter for ruminant rations (Miller *et al*., 1991), lower levels were found at the sites with low Co-content in grasses. Despite this, *Vicia sativa* will probably be able to increase the Co-content in the diet to some extent. Also, the appearance of *Alchemilla sp*. and *Rumex longifolius* in the meadows may be positive if there is deficit of Co.

In Study A, mean Cu:Mo-ratio in perennial grasses was above the recommended ratio of 6-10 (Frøslie and Nordheim, 1983), but in four of samples the ratio was below. In Study B the ratio was relatively low in all grass samples, and no significant differences between grasses and weeds were found. To elevate a low Cu:Mo-ratio *Lolium multiflorum* or *Raphanus sativus* seem to be appropriate species. Due to low Cu:Mo-ratios in *Vicia sativa* and *Pisum sativum* even at sites with a high ratio in perennial grasses, it will be useful to introduce one or both of the species into diets with high Cu:Mo-ratios.

No significant correlations between pH or contents of Co, Cu and Mo in the soil in one hand, and the mineral content in plants in the other, were found.

Table 1.Contents of cobalt (Co, mg $kg^{-1}$), copper (Cu, mg $kg^{-1}$) and molybdenum (Mo, mg $kg^{-1}$) in dry matter (DM) of green fodder crops, dicotyledonous weeds and perennial grasses from coastal areas in Norway. LS-means and probability value (p-value). Minimum and maximum levels for each species are given in brackets.

| | No | Co, mg $kg^{-1}$ DM | | Cu, mg $kg^{-1}$ DM | | Mo, mg $kg^{-1}$ DM | |
|---|---|---|---|---|---|---|---|
| **Study A** | | | | | | | |
| *Pisum sativum* | 5 | 0.04 | (<0.01 - 0.14) | 3.27 | (1.94 - 5.08) | 1.75 | ( 0.48 - 3.68) |
| *Vicia sativa* | 5 | 0.12 | ( 0.01 - 0.39) | 5.45 | (3.12 - 7.34) | 3.06 | ( 0.83 - 7.78) |
| *Avena sativa* | 6 | 0.05 | (<0.01 - 0.11) | 3.41 | (1.02 - 7.07) | 2.17 | ( 0.15 - 7.98) |
| *Hordeum vulgare* | 6 | 0.01 | (<0.01 - 0.03) | 2.72 | (1.56 - 4.25) | 0.67 | ( 0.09 - 2.26) |
| *Raphanus sativa* | 5 | 0.07 | (<0.01 - 0.09) | 2.74 | (1.65 - 4.52) | 0.94 | ( 0.08 - 1.69) |
| *Brassica napus* | 6 | 0.07 | (<0.01 - 0.26) | 2.92 | (1.67 - 5.46) | 1.80 | ( 0.07 - 4.04) |
| *Lolium multiflorum* | 6 | 0.08 | ( 0.01 - 0.20) | 3.78 | (1.95 - 6.28) | 0.70 | (<0.05 - 1.35) |
| Perennial grasses | 6 | 0.05 | (<0.01 - 0.10) | 3.10 | (1.90 - 4.76) | 0.67 | ( 0.04 - 1.21) |
| *p-value* | | 0.018 | | 0.018 | | 0.003 | |
| **Study B** | | | | | | | |
| *Alchemilla sp.* | 4 | 0.10 | ( 0.05 - 0.18) | 3.69 | (3.09 - 4.00) | 0.92 | (0.48 - 1.29) |
| *Rumex longifolius* | 4 | 0.10 | ( 0.05 - 0.15) | 5.91 | (5.34 - 6.47) | 1.12 | (0.72 - 1.36) |
| *Taraxacum sp.* | 4 | 0.02 | (<0.01 - 0.01) | 6.38 | (5.55 - 7.99) | 0.80 | (0.37 - 1.02) |
| *Anthriscus sylvestris* | 4 | 0.08 | (<0.01 - 0.24) | 8.03 | (6.58 - 9.70) | 1.09 | (0.44 - 2.02) |
| *Ranunculus repens* | 4 | 0.06 | ( 0.03 - 0.10) | 10.13 | (6.96 - 12.40) | 0.98 | (0.47 - 1.32) |
| *Festuca pratensis* | 4 | 0.03 | ( 0.02 - 0.06) | 5.18 | (3.79 - 5.78) | 0.91 | (0.43 - 1.25) |
| *Phleum pratense* | 3 | 0.01 | (<0.01 - 0.01) | 5.75 | (3.36 - 9.50) | 1.24 | (0.72 - 1.67) |
| *p-value* | | 0.056 | | 0.002 | | 1.695 | |

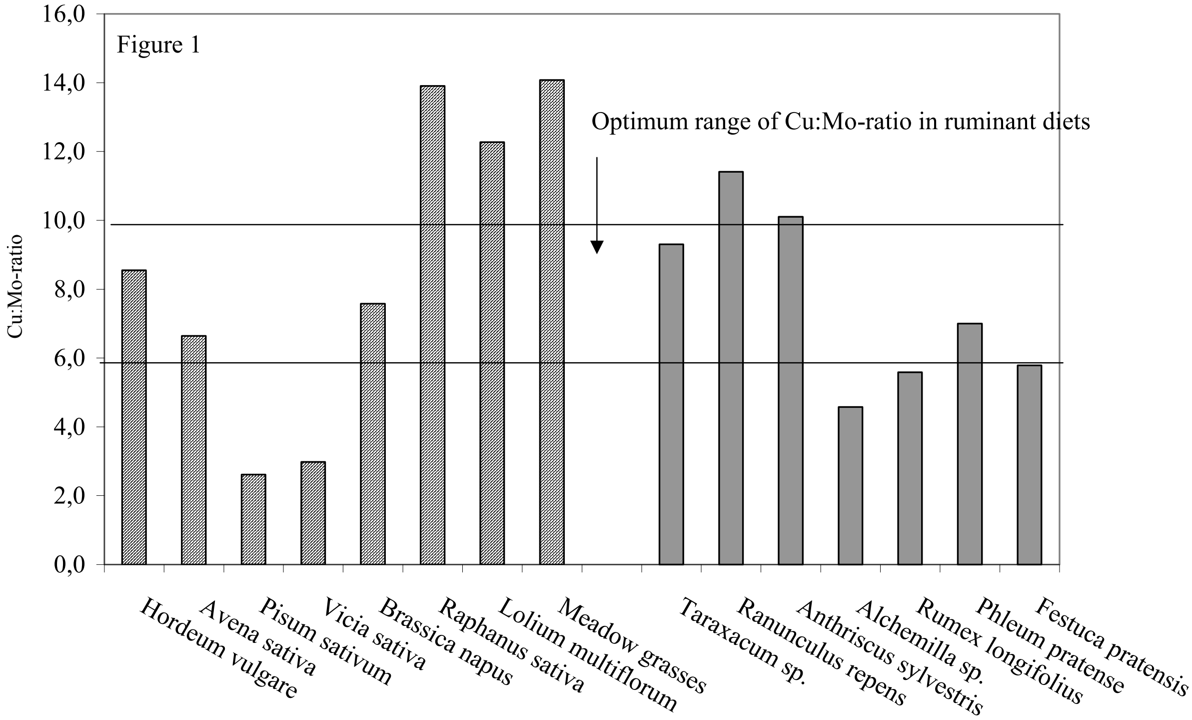


Figure 2. The ratio between copper and molybdenium (Cu:Mo-ratio) in dry matter (DM) of green fodder crops, dicotyledonous weeds and perennial grasses from coastal areas in Norway. LS-means. Horizontal lines gives the optimum range of the ratio in ruminant diets.

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# Manufacture of fertilizers containing micronutrients
# Possibilities and limitations

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**Fertilizer processes**

The most common methods for the production of fertilizers are 'Granulation' and 'Prilling'. The processes are characterized by the recycling of undersize and crushed oversize material that are mixed with fresh melt and dry salts, including micronutrients. The products are usually coated for the preservation of the quality in the handling chain.

There are several Granulation technologies (e.g. Drum, Pug-mill, Pan, Spherodizer, Fluidized bed), and in these processes the product must usually be dried in a hot drying drum after granulation. The recycled material regulates the granulation temperature, and the amount of recycle may be high compared to the feed melt. In the production period between two different grades there is a transition period where the recycle contains material from both grades. If micronutrients are added to the melt, they will also be spread through the whole process and may in this period be regarded as a contaminant, which can be of concern if the raw materials are not compatible with regard to safety.

In the Prilling process the mixture of melt, salts and recycled material is formed into droplets in a centrifuge or by using a nozzle-plate, and solidifies and cools when falling through the air in a prilling-tower. Micronutrients and other additives are usually added in a mixer just before prilling. The prilling-temperature may be higher than in granulation, because more water is removed for the droplets to solidify when falling through the tower. The amount of recycled material is usually lower in prilling than in granulation.

'Fattening' is a technique for depositing a layer of melt/coating on the surface on a fertilizer particle, and micronutrients can be added to the melt upon layering. The technique is commonly used for producing sulphur-coated urea and there are many ideas in the literature for binding various nutrients to the particle surface.

**Micronutrient additives**

Micronutrients are usually added in the fertilizer processes as dry salts, either as oxides or sulphates. The sulphates are water-soluble and are readily available for the plants, while the oxides are citrate soluble. Micronutrients can also be processed as water-soluble chelates where the cations are tied up to chelating agents. Chelates can be added directly to the process melt and will act efficiently with high water-solubility. However, chelates are more expensive than oxides or salts, and are less used in compound fertilizers. The amount of cations may typically vary in the range 0.01-0.2 % by weight in compound fertilizers.

The stability aspects are of crucial importance when designing and using products based on chelate chemistry, and factors like pH and heterogenic interactions must be taken into careful consideration. For example, Fe-EDTA is stable as long as the pH is kept on the acidic side, but as soon as a critical concentration of hydroxide ions is exceeded (at pH 6.5), the competing equilibrium of $Fe(OH)_3$ formation will "pump" iron out of the EDTA complex, forming precipitated $Fe(OH)_3$. At higher pH more stable Fe-chelates are needed.

**Possibilities in Dry applications**

Formulation wise there are three main principles for applying micronutrients with fertilizers in 'Dry applications':

- Incorporation of micronutrients in the melted stage of the manufacturing processes
- Coating on fertilizer prills/granules
- Dry blending

Incorporation in the melted stage is only economical if prepared in relatively large volumes. This again limits the number of product differentiations, so that such products are mostly designed for maintenance treatment on a quite general basis. There may be some variations in the availability of the micronutrients, depending on reactions in the process melt, dissolution rates and soil conditions. However, the products can be made homogeneous, and new formulations will be tested prior to the launching in the market, thus ensuring even spreading, application and uptake of the required (micro) nutrients.

Coating of micronutrients onto prills or granules may be advantageous over the two other principles; 1) Flexibility can be maintained through simple coating processes, and 2) the products can be made as homogeneous prills or granules, ensuring even spreading and application rates directly proportional to the macronutrient rates. The only pitfall is the binding of the micronutrients compound onto the granules, which may result in segregation of fines and caking of the product.

Blending is in some countries used to add specific amounts of micronutrients salts into the product mix. However, the potential in-homogeneity of the blends can often result in serious segregation and very uneven field application rates. For example, dry blending is common practice for correcting Zinc deficiency, and by blending 3 kg ZnO into 40 kg, not more than approximately 7 % of the field is treated with micronutrient, which could be improved by other application methods. The use of larger micronutrient particles will result in even more scattered application in the field.

**Solubles and liquids (fertigation and foliar)**

Formulation of soluble and liquid products for foliar, hydroponic or soil application needs some careful consideration which is not similarly important in dry applications: For foliar products factors like spreading, wetting and phyto-compatibility are vital in addition to nutrient composition.

**Liquid impregnation**

In 'Liquid impregnation' a liquid suspension of micronutrients is sprayed on the surface of the fertilizer particle. Yara International has carried out tests in cooperation with the micro-nutrient supplier Phosyn, and it has been shown that micronutrients can be layered on fertilizer particles. However, spraying NPK-fertilizers with water-based systems will tend to deteriorate the physical quality, and products from various processes will behave differently. Most impregnated NPK products will keep its quality in the short term but may deteriorate during long time storage, mainly because of crystallization between the fertilizer particles that will result in the generation of dust.

**Limitations**

There are limitations in many production processes to the use of micronutrients due to safety and the costs involved. NPK-products containing ammonium nitrate (AN) has the ability to decompose thermally if heated above e.g. 150 °C for some time, and some products may even decompose self-sustainingly (cigar-burning). Some micronutrients may act as catalysts for these chemical reactions, and accelerate the thermal decomposition of AN. If micronutrients are mixed, there may be a synergistic effect between the individual cations. Accordingly, the safety margins in the production plant will be reduced, and there is a particular safety concern if the product also contains chloride. Before launching a product on the market, the new formulation will be tested according to mandatory, official tests and by in-house test methods, thus ensuring that the product is stable in transport and handling.

The most hazardous formulation occurs if copper is added to a product containing chloride, and there have been severe accidents in the past with such compositions. A warehouse containing 7200 tonnes of Chloride based NPK 16-5-12 with 0.1 % Cu decomposed in Germany in December 1966, and the whole bulk was affected. Such a formulation is therefore not acceptable today. More recently an accident also took place during a transition from Chloride- to Sulphate-based NPK in a fertilizer plant based on the granulating technology. The main hazard in such decompositions is the spread of toxic gases, while the heat of reaction is normally too low to cause any harm outside the local area.

These safety limitations are not relevant to the same degree for products not containing AN, i.e. products based on MAP/DAP, superphosphates, urea. The international legislation for transport of storage of AN-products does not include micronutrients in the text. However, most producers are aware of the additional hazard, and may classify these fertilizers as ‘grey area’ products, depending of the content of additives and type of products.

The costs for producing fertilizers with micronutrients depend on the price of the raw materials, the available hoppers/belts for storing/handling the micronutrients and the volume to be produced. If the volumes are too small, the costs may be too high. In some cases the micronutrients may also discolour the product, which in some markets is undesired.

However, for most applications fertilizers can be designed to satisfy the demand from the customers to safe handling and at acceptable costs.

## High-throughput profiling of micronutrients and trace elements in crops for improvement of nutritional content and traceability

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Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is a unique and powerful technique for rapid simultaneous and sensitive analysis of more than 90% of the elements in the periodic table. Due to these analytical merits, ICP-MS is a useful functional genomics tool, allowing the connections between genes, proteins, metabolites and mineral ions in plants to be deciphered.

The ICP-MS at KVL is housed in newly constructed ISO-certified clean-room facilities and is equipped with an octopole reaction cell enabling accurate measurements at the ppt-level of even problematic elements such as Cr, Fe and Se in small quantities of tissue – a single *Arabidopsis* silique or aleuron cells from a few barley grains. Modern support equipment such as microwave oven, sub-boiling device for solvent clean-up and a Milli-Q element purification unit for ultra-clean water production is also available.

In combination with the ICP-MS we have developed an on-line system to simultaneously measure the uptake of all essential plants micronutrients down to the low nM concentration range. This system has been used to identify a new uptake system for Mn in plants and to study differences between barley genotypes in the capacity for Mn uptake and metabolism. We are also using the multi-elemental capabilities of ICP-MS in combination with chemometrics to analyse the elemental fingerprint that plants receive from the soil in which they grow. The obtained fingerprints allow accurate prediction of the geographical origin of different barley genotypes, demonstrating a major potential in studying traceability of plant food products. The future work will include novel essential plant nutrients, the so-called nanonutrients, consisting of, e.g., rare earth elements (REE) and platinium group elements (PGE).

Combining ICP-MS with various chromatographic techniques such as HPLC, FPLC and IC (LC-ICP-MS) offers a lot of exciting possibilities in plant science because the organic speciation of the elements often are much more biological relevant than just the total tissue concentration of a given element. We are currently using HPLC-ICP-MS to study Cd scavenging peptide complexes in plants. Furthermore, the distribution and speciation of Fe and Zn in different tissues of the cereal grain are explored. The results these different projects will be presented.

# Soil analysis: key to nutrient management planning

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## Introduction

Any definition of sustainability related to the managed use of land must include physical, environmental and socioeconomic aspects. No agricultural system will be sustainable if it is not economically viable both for the farmer and the society of which he is a part. But economic sustainability cannot be bought at the cost of environmental damage that is ecologically, socially or legally unacceptable, or physical damage that leads to irreversible soil degradation or uncontrollable outbreaks of pests, weeds and diseases.

In many parts of Northern Europe and Scandinavia considerable intensification of both crop and animal farming systems has taken place. Initially after the Second World War the government of most countries actively encouraged their farmers to increase agricultural productivity to secure the country's food supply. Then more recently, farmers themselves have sought to increase their yields to maintain the economic viability of their farms in the face of ever decreasing prices of food products on world markets.

Many factors have contributed to the increases in productivity. They include the ready availability of plant nutrients, especially nitrogen (N), as fertilizers, the introduction of cultivars of many crops with a large yield potential, the ability to protect that potential by controlling pests, diseases and weeds and farmers' management skills to use these improvements effectively. It is unfortunate that the occasional, perhaps unwitting, overuse of some of these inputs in some farming scenarios has had adverse environmental impact, sometimes outside the soil-plant-animal system, sometimes within it. With the increasing realization of risk in some situations, remedies have to be sought if farming systems are to remain sustainable.

## Nutrient management

Currently in many countries in Europe and Scandinavia, farmers are being encouraged, or are required to prepare and maintain nutrient management plans for their farms. The underlying purpose would seem to be to minimize the risk of loss of nutrients, especially N and phosphorus (P), from the soil-plant-animal system to the wider environment. Whilst this is laudable in itself, it is essential that if the amount of each nutrient that can be applied to a particular crop in a specific farming system is to be regulated, then such regulation should not put at risk producing optimum crop yields of acceptable quality. This is important because on this depends the financial viability both of the farms themselves and the wider community dependent on them. Equally important however, the imposition of nutrient management plans and the regulation of nutrient inputs should not put long-term soil fertility at risk. If nutrient management plans are intended to minimize nutrient losses then the essential need for nutrients in food production must be assessed against any associated risk of their transfer to other parts of the environment and any adverse effect they may have there. It is important that any risk is targeted accurately and with least detriment or cost to achieving either objective.

In principle integrated plant nutrient management is to be applauded for it should maximize profit from the efficient use of inputs as well as minimize nutrient transfers from agricultural soils to the environment. In practice this might not be achieved easily because we are dealing with a biological system subject to many variables where soil processes mediated by the living biota in soil can vary the amount of nutrients required by plants.

Integrated plant nutrient management requires farmers to consider all sources of nutrients available to a crop to be grown on a specific field. So first there will be the availability of nutrients already in the soil and it is in this context that soil analysis is important. Then there may be organic manures produced on the farm, like slurry, or purchased, like sewage sludge, and these must be used efficiently. Once the quantity of nutrients available from these sources has been estimated, the balance to be applied as fertilizers to meet the requirement of the crop can be calculated and a nutrient management plan drawn up.

**Soil analysis**

Turning to soil analysis, it has limitations as well as possibilities. Most widely used methods of soil analysis have “stood the test of time” so that getting a representative sample is essential. A whole field sample is acceptable provided the field is uniform, otherwise areas distinctly different in soil texture, topography or known yield potential should be sampled separately. Each sample should consist of 25 cores bulked together. When grid sampling, 16 cores should be taken at each sampling point.

When considering soil sampling it is necessary to consider the function of individual nutrients in relation to crop production and soil fertility. For example, we should consider N differently from P and potassium (K). Rarely does sufficient N accumulate in soil in plant available forms to meet the requirement of crops with a large yield potential and, in consequence, supplementary N has to be applied each year. Soil organic matter holds the main reserve of N in many soils but this N is not immediately available to plants. On the other hand, plant available P and K can accumulate in many soils in sufficient quantities to meet the needs of crops and these reserves can be measured by soil analysis.

For N, soil analysis measures the mineral N, usually to 90 cm, and often seeks to estimate the N in surface soils that might become available by mineralization of soil organic matter during the growing season. In England and Wales soil analysis for N is frequently advised only where there might be large quantities of mineral N in spring following a previous crop, like potatoes or vegetables, to which much N was applied.

For P and K, plant available reserves can be estimated by suitable methods of analysis. From the relationship between yield and soil analytical values, a critical value for soil plant available P and K can be determined. Below the critical value yields are less than the potential optimum and farmers suffer a financial penalty. Maintaining soils above the critical value is an unnecessary expense for the farmer and there is a risk with soils over enriched with P that if soil is eroded into surface water then there may be a penalty to society from the adverse effects of eutrophication. Similar environmental issues do not arise with K although there is still a financial cost to the farmer if soils are maintained much above the critical value. Thus nutrient management plans for P and K should aim to first raise soil values to just above the critical value and then maintain this value by at least replacing the P and K removed from the field in the harvested crop.

The ability to define critical values for plant available P and K by soil analysis shows that this approach is valid for those nutrients that can be accumulated as soil reserves. It could act, therefore, as a template for assessing the needs of micronutrients but this will not be easy. Additionally correcting deficiencies may be done better by foliar sprays rather than by accumulating reserves from soil applications.

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# Zinc reduces secretion from piglet small intestinal epithelium

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**Introduction**
Dietary zinc reduces diarrhoea in weaned piglets and in undernourished children (Poulsen, 1995; Fuchs, 1998). Furthermore, oral rehydration solutions (ORS) containing zinc have been shown to be efficient in controlling acute diarrhoea in children in developing countries (Bahl et al., 2002). The mechanisms behind this effect have not yet been fully established. However, Ussing chamber studies showed that high levels of dietary zinc (2500 mg/kg) to weaned piglets reduced the electrophysiological responses to secretagogues in the small intestinal epithelium (Carlson et al., 2004). However, it is unclear if this effect of zinc oxide is a direct antisecretory effect of zinc ions in the epithelial tissue, or if it is an indirect effect of e.g. a more healthy epithelial tissue (Li et al., 2001). Consequently, the aim of the present study was to study if zinc per se has a direct and immediate anti-secretory effect in intestinal epithelium from weaned piglets.

**Materials and methods**
Exp. 1 and 2 included 12 piglets receiving a post weaning diet containing 100 ppm zinc (from ZnO). Exp. 3 included 24 piglets that were allocated on two post-weaning diets (100 or 2500 ppm zinc, from ZnO). All the piglets were slaughtered at 5-6 days after weaning. Immediately after slaughter, samples of the small intestinal epithelium were mounted in Ussing chambers. In the Ussing chambers the effect of zinc (ZnSO4) in the bathing media (either on the mucosal, serosal or both sides) on electrophysiological responses to 5 different secretagogues was studied. The size of the responses (measured as increase in short circuit current, ΔIsc) corresponds to the magnitude of secretion, of mainly chloride, induced by the secretagogues.

**Results and discussion**
In the following, some of the preliminary results from the experiments are presented. The effect of zinc on the secretory response to serotonin (5-HT) and theophylline is shown in Fig.1. It is clear that zinc had a concentration dependent influence on the response to 5-HT, with the lowest response when the zinc concentration was equal to or higher than 0.023 mmol/l. The response to theophylline was however, not affected by zinc. On the background of these results it was chosen to use a zinc concentration of 0.023 mmol/l in the following experiments.

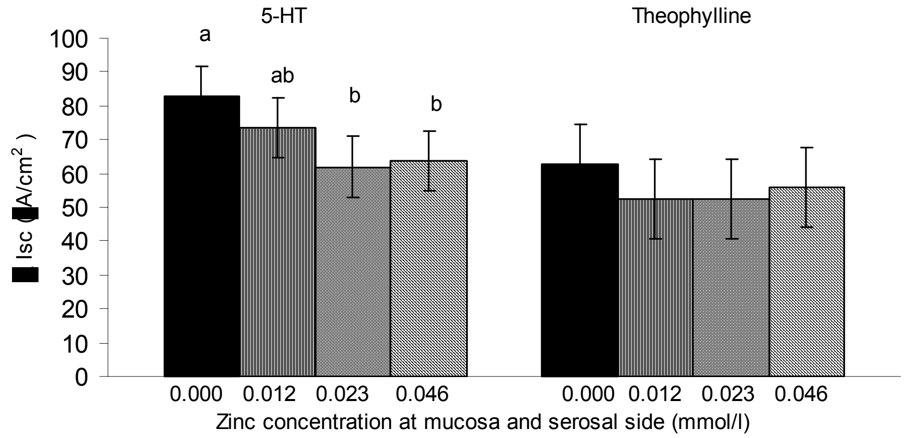


Fig. 1. The epithelial response (increase in short circuit current, ΔIsc) to serotonin (5-HT) and Theophylline when the intestinal epithelium from piglets fed 100 ppm zinc was bathed in increasing zinc concentrations (Exp.1). Results are least square means and SE (N=12 piglets). Values with different letters within the same secretagogue differ significantly (p≤0.05).

The effect of zinc on the epithelial responses to 4 different secretagogues in study 2 is illustrated in Fig. 2. It shows a significant reducing effect of 0.023 mmol/l of zinc on the responses to vasoactive intestinal peptide (VIP) and carbachol. However, the responses to substance P and theophylline were not affected by this concentration of zinc. The dissimilarity between the effects of zinc on the secretagogues may illustrate the different pathways through which these secretagogues act. The results shown in Fig. 1 and Fig. 2 indicate that the pathways through which 5-HT, VIP and carbachol acts were at some stage affected by the zinc ions in the bathing media.

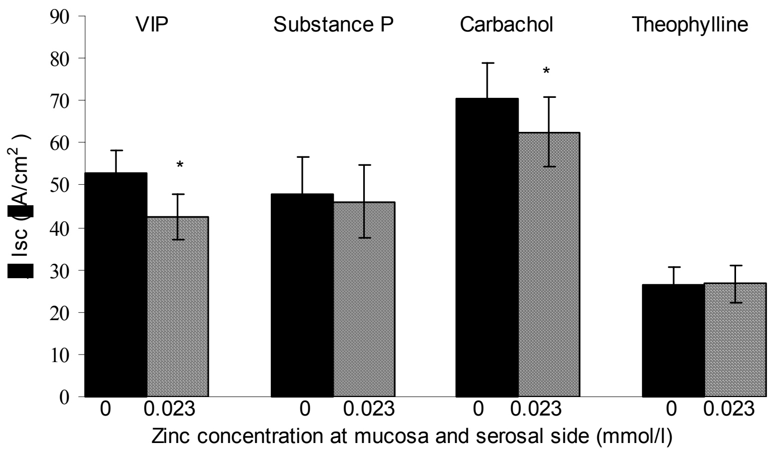


Fig. 2. The epithelial response (increase in short circuit current, ΔIsc) to vasoactive intestinal peptide (VIP), Substance P, Carbachol and Theophylline, when the intestinal epithelium from piglets fed 100 ppm zinc was bathed in 0 or 0.023 mmol/l of zinc (Exp.2). Results are least square means and SE (N=12 piglets). * indicate significantly different responses to the same secretagogue (p ≤ 0.05).

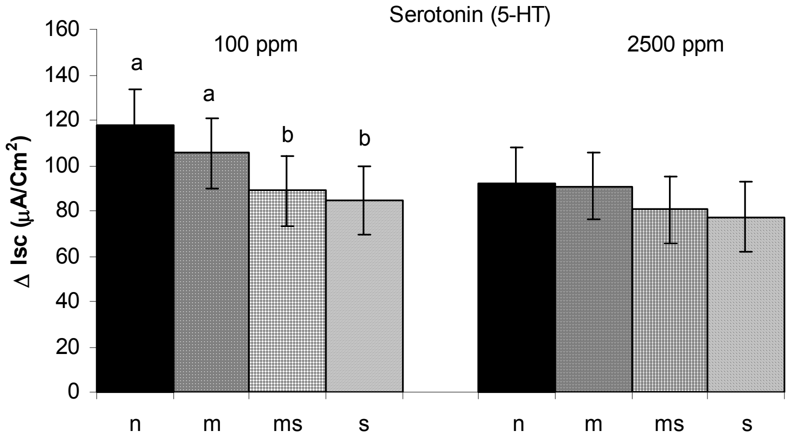


Fig. 3. Epithelial response (increase in short circuit current, ΔIsc) to 5-HT when piglets had been fed 100 or 2500 ppm of dietary zinc and when the intestinal epithelium was bathed in a zinc free media (n) or in 0.023 mmol/l zinc at the mucosal (m), serosal (s) or both sides (ms) (Exp.3). For the 100 ppm group $p \leq 0.0001$ and for the 2500 ppm group $p=0.35$. There was a significantly lower ($p \leq 0.05$) response to 5-HT when piglets were fed 2500 ppm zinc compared to 100 ppm zinc. Results are least square means and SE (N=12 piglets in each dietary group). Values with different letters within the same secretagogue differ significantly ($p \leq 0.05$).

The results from exp. 3 showed that the VIP response was not affected by the dietary treatments (data not shown), but the response to 5-HT was reduced when piglets had consumed a diet containing 2500 ppm zinc for 5-6 days after weaning (Fig. 3). Further, it was found that zinc only had the attenuating effect on the 5-HT and VIP response (data for VIP not shown) when it was present at the serosal side of the epithelium. The latter indicate that zinc should be transported over the intestinal epithelium before it has the anti-secretory effect in vivo. Finally, the results from exp. 3 showed that the acute and direct effect of zinc at the serosal side in Ussing chamber was absent when piglets had consumed the high zinc concentrations after weaning.

## Conclusion

It is concluded that zinc ions at the serosal have a direct and immediate attenuating effect on the actions of some secretagogues in the intestinal epithelium. Further, the results suggest that the positive effect of dietary zinc on post weaning diarrhoea in piglets is associated with an anti-secretory effect of zinc ions in the epithelium.

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# Changes in the trace element contents of cereal products and vegetables in three decades in Finland

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## Introduction

The mineral and trace element contents of Finnish foods were analysed extensively in the department of Food Chemistry and Technology during 1970s. During the last 30 years many practices in agriculture have changed. The amount of used main plant nutrients has decreased by 25 %. The use of phosphorous (P) has decreased by 66 % since the year 1975 (Yearbook of Farm Statistics, 1983, 2003). The varieties of cereals have changed completely since 1975. Also a lot of new varieties of vegetables have emerged whereas the growing of old ones has ended or decreased significantly (Kinanen, 1981; Kangas & Teräväinen, 2004). The variety of plants, soil conditions, the use of fertilizers, and the degree of plants maturity at harvest is known to affect the mineral concentration of plants (Koivistoinen, 1980). Se supplementation of fertilizers has changed the Se content of all Finnish vegetable foods (Ekholm, 1997). Also the geological origin has an effect on minerals in foodstuffs, because the mineral contents vary in different countries (Sanchez-Castillo et al 1998).

For these reasons there was a need to update the trace element contents of Finnish vegetables as well as cereals and cereal products. In this study the contents of nine essential or potentially essential trace elements: aluminium (Al), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn), as well as two toxic elements: cadmium (Cd) and lead (Pb) were determined from 18 cereal or cereal products, 10 root vegetables, and 15 vegetable samples.

## Materials and methods

The samples were collected in a research project considering bioactive phenol compounds in the Forssa area in south Finland. All sub samples were combined to make one analysable sample per food item. All samples were freeze dried and homogenised. The organic matter of all samples was digested by wet ashing by concentrated nitrogen acid. The mineral and trace element contents except Se were determined with ICP-MS equipment. Se was analysed by an electrothermal atomic absorption method. All determinations were made triplicate.

The results were standardised for eliminating the analytical differences between the present study and the Finnish mineral element study (Koivistoinen, 1980). This was made by using one shared sample in both studies (wheat flour control sample), and calculating the relative contents of every mineral and trace elements determined in the present study.

## Results and discussion

The contents of trace elements were found to differ from the Finnish results reported by Koivistoinen (1980) (Fig 1-3). The contents of most elements had decreased. In general the decrease is 20% or smaller, but the contents of Fe, Al, Co and Ni in cereals were found to be more than half of the level measured in the1970's. In the root vegetables and green vegetables the contents of Mn, Zn, Al, Pb and Ni had decreased significantly, and in the vegetables also the contents of Cd and Co were decreased. The content of Se had increased in all food groups. Also the content of Co had increased in root vegetables. The contents of Cu in root vegetables were at the same level as in the 1970's. Also the contents of Fe in root vegetables and green vegetables had remained almost unchanged. The change in the mineral and trace element contents were greatest in radishes.

Consequently, the trace element density of cereals and vegetable foods is now lower than 30 years ago. Some of these changes have clear explanation. The Fe supplementation of wheat flour was omitted in 1994 and this naturally has decreased the Fe content in the cereal group. The emissions of Pb from traffic have decreased enormously during the last 30 years, because of the use of unleaded fuels. The average Pb content of food group had decreased. However, the average Pb content of cereals was increased. Instead the dry matter content of samples has not changed. Only radishes and celery now have clearly less dry matter than earlier. The use of the Se supplemented fertilizers was started in 1985 in Finland, and the Se contents of all Finnish foods have increased significantly since then. The main reason for these changes may, however, be the other varieties of cultivated plants, the decreased use of plant nutrients, and the increased use of imported food items. The samples of the Finnish mineral element study in the 1970's were almost completely domestic, but in the present study the amount of imported food items were significant.

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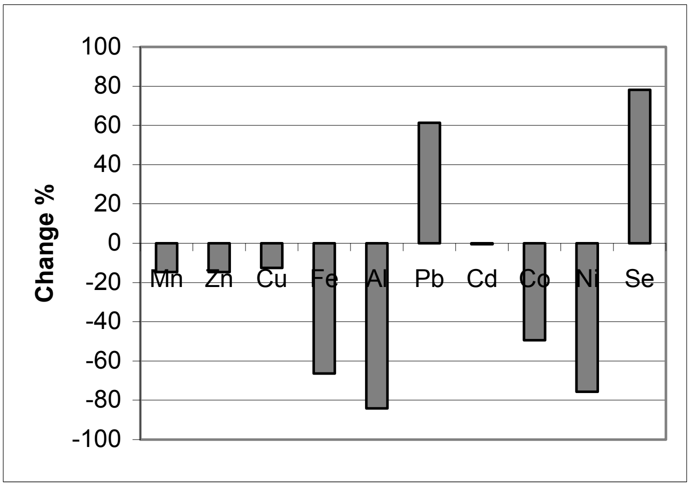


Fig 1. Changes of the average trace elements contents of cereals during the last 30 years.

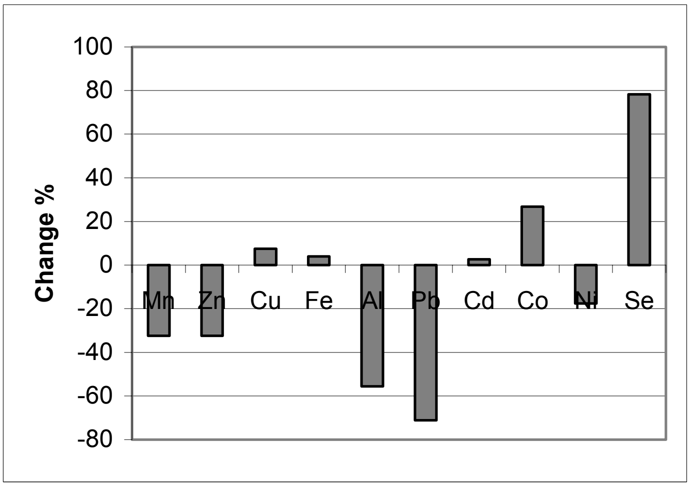


Fig 2. Changes of the average trace elements contents of root vegetables during the last 30 years.

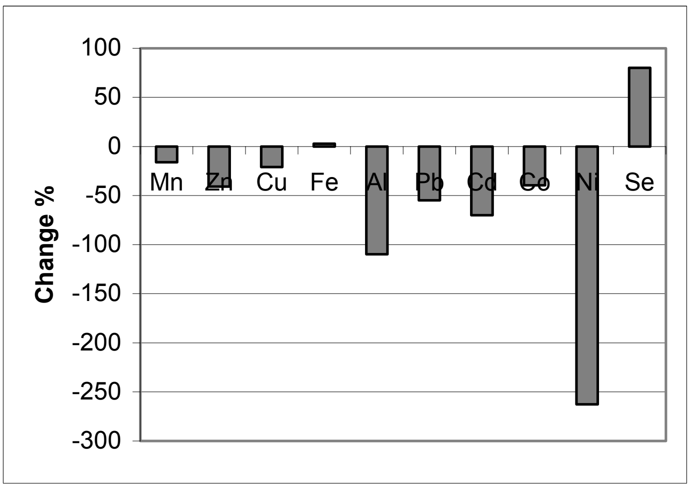


Fig 3. Changes of the average trace elements contents of green vegetables during the last 30 years.

# Trace elements and heavy metals in a long term field trial on an Andic Gleysol in Iceland

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## Introduction

A long term field trial using different N fertilizers was conducted for 43 years (1954 to 1996) on an andic gleysol at Skriðuklaustur in East Iceland. Annual mean temperature is 4.1°C and annual precipitation 501 mm. All plots received 74.4 kg K/ha and 30.6 kg P/ha annually. The pH in the top 5 cm changed from about 6.5 at the beginning of the experiment to 3.8 where $(NH_4)_2SO_4$ was used and to 5.8 and 6.9 where $NH_4NO_3$ and $Ca(NO_3)_2$ were used respectively. The organic C increased in all plots from 1973 to 1996 in the top 0-5 cm of the soil from 6.9-8.8% to 12.4-21.1%. At 5-10 cm depth C was 4.2-5.0% in 1973 and increased to 4.9-6.2% in 1996, except 7.9% with $(NH_4)_2SO_4$. At 10-20 cm depth C was about 4% and the increase between 1973 and 1996 was ≤1.0%. We report on the elemental composition of samples from 0-5, 5-10 and 10-20 cm depth from single plots with 120 kg N/ha as $NH_4NO_3$, $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ and an unfertilized area beside the experiments. A total of 12 samples were analysed.

## Results and discussion

The major and trace elements were measured by Plasma emission spectrometry (ICPAES) and Plasma mass spectrometer (ICPQMS) after melting 0.125g sample with 0.375g $LiBO_2$ and dissolving in $HNO_3$. Results are shown on mineral basis in Table 1, 3 and 4 but on oven dry soil basis in Table 2 and 5 and in Fig. 1. In studies of soil formation the elements Ti, Zr and V are often regarded as stable and are referred to as stable elements in the following discussion.

In Icelandic basalt a common range in Si, Al, Fe and Mn is 260-280, 69-85, 70-110 and 1.2-2.0 g/kg respectively (Jakobsson 1980). The soil values show a relatively high Si content on mineral basis, indicating a small admixture of rhyolitic ash. Otherwise the parent material has the characteristics of basalt. Ti in Icelandic basalt has a wide range of 3-34 g/kg compared to 1.8-2.2 g/kg in rhyolite. The Ti content is relatively stable, 16-18 mg/kg on mineral basis. However the other stable elements are more variable. On mineral basis Zr is lowest in the top 5 cm of the soil and much lower in the acid plots than in other plots, indicating that it has been leached from this layer, but does not show any trend related to the

Table 1. The main elements Si, Al, Fe, Mn, S and Ti calculated on mineral basis. Mean values and range in the plots g/kg.

| Depth cm | Si | Al | Fe | Mn | S | Ti |
|---|---|---|---|---|---|---|
| 0 -5 | 289<br>281-308 | 71<br>62-77 | 98<br>94-101 | 1.9<br>1.1-2.5 | 3.5<br>1.8-6.7 | 17<br>16-18 |
| 5 -10 | 292<br>290-295 | 78<br>77-81 | 104<br>100-108 | 2.0<br>1.7-2.6 | 1.3<br>0.6-2.5 | 17<br>17-17 |
| 10 -20 | 294<br>289-299 | 78<br>74-82 | 105<br>100-111 | 1.9<br>1.6-2.2 | 0.7<br>0.5-0.9 | 17<br>16-18 |

Table 2. Total trace element composition in oven dry soil and common range of threshold values in some EU countries, mg/kg oven dry soil.

| | As | Ba | Be | Cd | Co | Cr | Cu | Hg | La | Mo | Nb | Ni | Pb | Sc | Sn | Sr | V | W | Y | Zn | Zr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 4,5 | 121 | 1,1 | 0,36 | 18 | 77 | 50 | 0,06 | 10 | <6 | 12 | 17 | 3,5 | 28 | <20 | 161 | 279 | <60 | 35 | 56 | 164 |
| Max | 12,5 | 145 | 1,4 | 1,22 | 27 | 95 | 60 | 0,19 | 16 | <6 | 17 | 21 | 5,4 | 34 | <20 | 250 | 324 | <60 | 40 | 66 | 200 |
| Min | 0,6 | 55 | <0,6 | 0,09 | 3 | 61 | 33 | <0,04 | 7 | <5 | 6 | 6 | 1,5 | 16 | <20 | 70 | 176 | <50 | 20 | 23 | 56 |
| Threshold values | | | | 0,4-1,5 | | 30-100 | 20-60 | 0,1-1,0 | | | | 15-70 | 40-100 | | | | | | | 60-200 | |

location of plots (Table 3). The stable element V, on the other hand, shows clear trend on mineral basis. It is 383 mg/kg in the top 5 cm and decreases to 332 mg/kg at 10-20 cm depth. Also, within the area, the mean values, mean of three depths, decrease continuously from 392 mg/kg in the plot with $NH_4NO_3$ to 324 mg/kg in the unfertilized area (Table 3). This could indicate a trend in the parent material within the experimental field. Alternatively these trends could be associated with the fertilization as the vanadium is highest in the top 5 cm of the soil and lowest in the unfertilized area. The low mean value for Al in the top 5 cm of the soil is due to low content in the acid plot Considering the narrow range for the stable elements Ti and Zr and for Si, and Al except in the top 5 cm of the acid plot, it is concluded that the parent material is fairly homogeneous and that variations in other elements can be interpreted as a result of the fertilizer treatment. The slight rise in Fe with depth may be explained by the gleyic properties of the soil and precipitation of iron.

Table 3. Mean values for Ti, V and Zr for the three depths. Ti in g/kg; V and Zr in mg/kg.

| Treatment | Ti | V | Zr |
|---|---|---|---|
| **$NH_4NO_3$** | 18 | 392 | 206 |
| **$(NH_4)_2SO_4$** | 17 | 371 | 190 |
| **$Ca(NO_3)_2$** | 16 | 343 | 205 |
| **No fertil.** | 17 | 324 | 215 |

Most of the elements are within the range that can be expected for basalt. No change with depth or variation between plots was found for La, Nb, Sr, and Y. When calculated on whole soil basis the variation in the contents observed can basically be explained as dilution by organic matter of the mineral born elements. This is not the case for Cr, Cu, Pb, Sr, Zn and V that are highest in the top 5 cm of the soil (Table 3 and 4). Apparently these elements have been brought into the soil, most probably through the fertilizer as the lowest values are in the unfertilized area.

A number of elements, Ba, Co, Ni and Zn, are lowest in the top 5 cm of the acid plot ($(NH_4)_2SO_4$) indicating increased leaching of these elements upon the extreme acidification (Table 4). These are the only micronutrients that show clear signs of depletion. The low values in the acid plot are responsible for the lower mean values of Ba and Co in the top 5 cm compared with the other depths (Table 4). However the mean value for Ni is not affected and Zn is on average higher in the top 5 cm than at greater depth indicating input through fertilization.

Table 4. The depth function of some trace elements. Mean values in oven dry soil mg/kg.

| Depth cm | Ba | Co | Cr | Cu | Ni | Pb | Sr | Zn |
|---|---|---|---|---|---|---|---|---|
| 0 -5 | 144 | 19 | 113 | 68 | 21 | 7,5 | 241 | 78 |
| 5 -10 | 159 | 25 | 91 | 63 | 20 | 3,3 | 195 | 67 |
| 10 -20 | 155 | 25 | 94 | 61 | 22 | 3,6 | 182 | 69 |

The contaminating elements, Cd and Hg, are constantly highest in the top 5 cm of the soil and lower in the plot without fertilizer (Table 5). These elements have been applied with the fertilizers and they have accumulated in the top few cm of the soil. In the ammonium nitrate and calcium nitrate plots the Cd enrichment is mostly confined to the top 5 cm but in the acid ammonium sulphate plot it has moved down so that it is even found at 10-20 cm depth. Mercury is known to be strongly bound to the organic substance and its enrichment is confined to the top 5 cm. Both elements reach or are slightly above the lowest threshold values used in some EU countries.

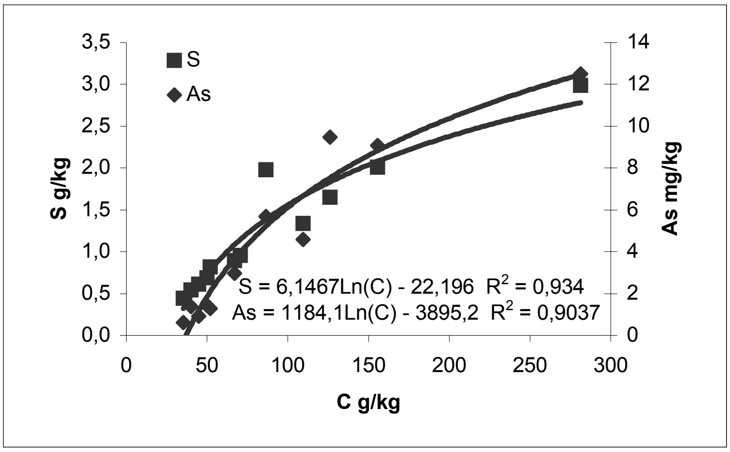
Table 5. Cadmium and mercury. Mean values in oven dry soil mg/kg.

| Depth cm | $NH_4NO_3$ | | $(NH_4)_2SO_4$ | | $Ca(NO_3)_2$ | | No fertil. | |
|---|---|---|---|---|---|---|---|---|
| | Cd | Hg | Cd | Hg | Cd | Hg | Cd | Hg |
| 0 -5 | 0.88 | 0.12 | 0.55 | 0.18 | 1.22 | 0.19 | 0.26 | 0.09 |
| 5 -10 | 0,15 | 0.05 | 0.45 | 0.05 | 0.28 | 0.05 | 0.09 | <0.04 |
| 10 -20 | 0,11 | <0.04 | 0.24 | <0.04 | 0.11 | 0.04 | 0.10 | <0.04 |

The sulphur content is highest in the top 5 cm and decreases with increasing depth. The highest S contents are found in the plot which received ammonium sulphate as N fertilizer.

Arsenic and sulphur are highly correlated with the C content (Fig. 1). The correlation can be described by a logarithmic function where $R^2$ is 0.93 for sulphur and 0.90 for arsenic. The As content of the top 5 cm of the fertilized plots ranges from 9.1 ($Ca(NO_3)_2$) to 12.5 mg/kg ($(NH_4)_2SO_4$) compared with 4.6 mg/kg in the top 5 cm of the unfertilized area. As the arsenic content of basalt is in the range of 1.5-2.0 mg/kg and values of that range are found at 10-20 cm depth it appears that there has been some As enrichment especially in the fertilized plots. In the EU threshold values for As have not been introduced but are likely to be around 20 mg/kg. According to the German federal soil protection law grassland has to be evaluated when As values reach 50 mg/kg.

Figure 1. Correlation between total contents of C and S and C and As. n=12.




**Summary**

The elemental composition is in a range characteristic for basalt and none of the micronutrients can be considered as low. The lowest contents of the micronutrients Ba, Co and Zn in the acid plots show the importance of controlling pH to avoid leaching. Both Fe and Mn are slightly lower in the acid plot also indicating some leaching of these elements. Together with S the micronutrients Cr, Cu and Zn are generally highest in the top 5 cm of the soil in the fertilized plots indicating that they have been brought in with the fertilizer.

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# Molybdenum and sulphur in forage samples from scrapie-free, scrapie-prone and scrapie-afflicted farms in Iceland

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## Introduction

It has been postulated that low levels of copper in herbage are connected with the occurrence of scrapie in Iceland (Purdey 2000). Although previous research by the authors does not support this postulate (Jóhannesson *et al.* 2004a), it is possible that molybdenum in the forage, especially with high amounts of sulphur, could result in formation of copper chelates and thereby interfere with the bioavailability of copper, promoting occurrence of clinical scrapie. Forage samples (round bale silage, hay) were therefore analysed for molybdenum (Mo) and sulphur (S) concentrations in order to study whether occurrence of clinical scrapie in sheep could be related to levels of these nutrients in forage. In order to calculate the copper/molybdenum (Cu/Mo) ratios, results of copper analyses in the same forage samples, previously published by the authors, were also included (Jóhannesson *et al.* 2004a).

## Materials and methods

Forage samples (in total 110), from the summer harvest in 2003, were collected on 36 sheep farms for analysis of molybdenum and sulphur. Farms were divided into three categories. *Scrapie- free*: Twenty-two farms never afflicted by scrapie, or afflicted and restocked with healthy sheep prior to 1960. *Scrapie-prone*: Seven farms afflicted by scrapie after 1980 and afterwards restocked with healthy sheep. *Scrapie-afflicted*: Seven farms where scrapie was diagnosed during the experimental period (summer 2002 – March 2004). Molybdenum was determined in the samples by graphite furnace atomic absorption spectrometry and sulphur by ICP optical emission spectrometry.

## Results

Molybdenum concentration varied greatly in the forage samples, especially in forage samples from scrapie-free and scrapie-afflicted farms (Fig.1). The high levels of molybdenum in individual samples were ascribed to haphazard presence of plants (or parts of plants) containing more than average amounts of molybdenum. Sulphur concentration was remarkably constant in forage samples from farms in all categories (Fig.2). Cu/Mo ratios were most often in the interval 10-200, the lowest being about 6 (Fig.3).

## Conclusions

It was concluded that the levels of molybdenum and sulphur in the forage for sheep are not directly connected to occurrence of clinical scrapie. Although sulphur may interfere with the availability of selenium it is doubtful whether the levels of sulphur observed may explain the widespread selenium deficiency found in forage of sheep in Iceland (Jóhannesson *et al.* 2004b). The levels of molybdenum in forage of sheep are not likely to interfere with the availability of copper as the critical level for occurrence of such interferences is at Cu/Mo ratios lower than 4 (Adriano 2001).

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Figure 1. Collective distribution of individual results of molybdenum determination in forage samples from scrapie-free, scrapie-prone and scrapie-afflicted farms.

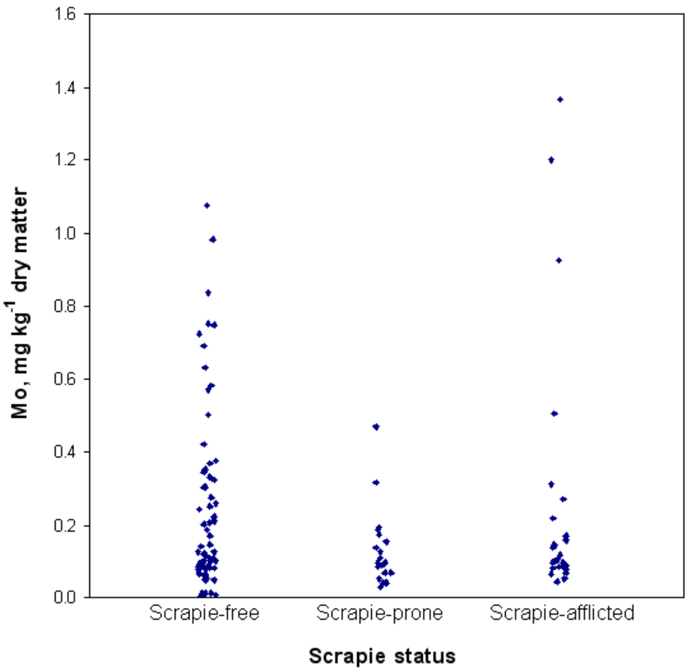


Figure 2. Collective distribution of individual results of sulphur determination in forage samples from scrapie-free, scrapie-prone and scrapie-afflicted farms.

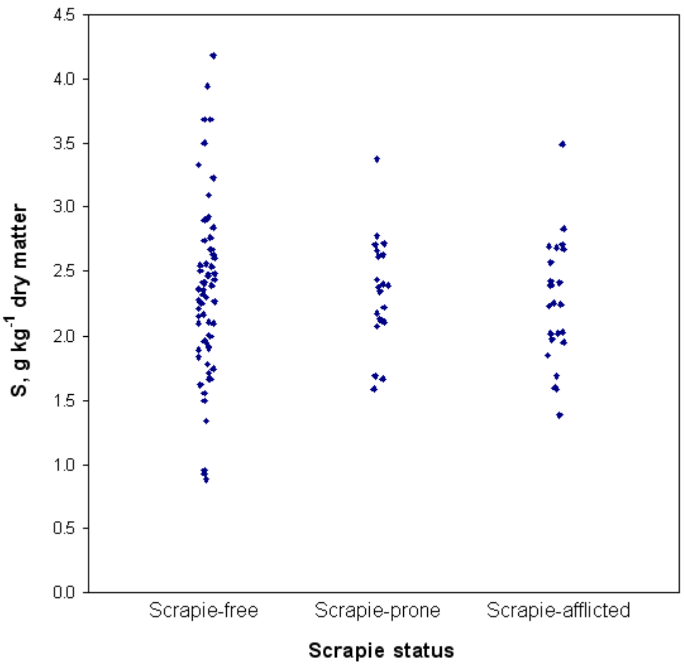
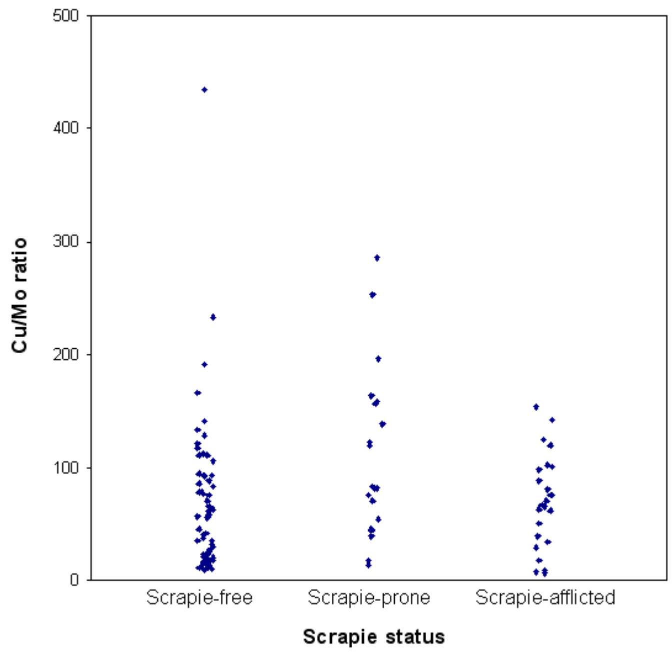



Figure 3. Collective distribution of individual Cu/Mo ratios in hay samples from scrapie-free, scrapie-prone and scrapie-afflicted farms. Three individual very high Cu/Mo ratios were excluded as they fell above the frame of the figure.



# Selenium status in Icelandic horses assessed indirectly

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## Introduction

Selenium is an essential nutrient for animals, although toxic at higher concentrations (Schwarz and Foltz, 1957). Selenium is an important component of the enzyme, glutathione peroxidase (GSH-Px) (Rotruck, et.al, 1973) and, in conjunction with vitamin E, this is an essential protection from oxidative damage to body tissues (Lewis, 1995; Blood and Radostits, 1989). The clinical signs of deficiency of selenium and/or E-vitamin in farm-animals include stiff and weak muscles, known as white muscle disease (Lewis, 1995).

Selenium-deficiency is commonly observed in Icelandic lambs (Johannesson et al, 2004) and is known in other farm-animals (Olafsson et al, 1999). In addition, GSH-Px activity in sheep is lower after winter feeding than after summer grazing in highland pastures (Johannesson et al, 2004) because selenium concentration in hay in Iceland seems to be low or very low (Johannesson et al, 2004).

GSH-Px activity is directly related to selenium levels in the blood of farm-animals (Blood and Radostits, 1989, after Koller and Exon, 1986). Therefore, the selenium status of farm-animals in Iceland has been assessed indirectly by measuring the activity of the enzyme in whole blood expressed as units/gr Hemoglobin (U/gr Hb) (Olafsson et al, 1999, Arnthorsdottir, 2002, Johannesson et al, 2004)

Sources give different reference values of GSH-Px activity for horses. At the Institute of Experimental Pathology, University of Iceland, Keldur, Reykjavik it is expected that values of GSH-Px activity in horses ≤ 100 U/gr Hb indicate selenium deficiency, and this is in accordance with the manual from Randox Laboratories Ltd UK. Kaneko et al, 1997 reported 79,31 ± 16,20 U/gr Hb as reference value for GSH-Px activity in whole blood of horses. Blood and Radostits (1989) reported GSH-Px activity in horses between 30-150 µmol/min at 37°C/g Hb (comparable to 30-150 U/gr Hb), and suggested that values between 8-30 µmol/min at 37°C/g Hb (comparable to 8-30 U/gr Hb) indicated selenium deficiency. However, the relationship among antioxidants in the body of horses is complicated, e.g. high levels of vitaminE can partly compensate for low level of selenium. Therefore, it is difficult to give exact reference values of GSH-Px (Blood and Radostits, 1989 and Kaneko et al, 1997).

## Materials and methods

Available data on measurements of GSH-Px in horses, from the years 1999-2005, were collected, at the Institute of Experimental Pathology, University of Iceland, Keldur, Reykjavik. These data are in total blood-samples from 70 horses. Repeated blood samples were available from seven of the horses taken 8 weeks after the initial sample. It should be emphasized, that these 77 measurements were not collected in a systematic way, but were measurements from blood-samples which were occasionally sent in by veterinarians for different reasons to assess the selenium status. No further information was available on the health status of the horses or if they showed signs of selenium-deficiency. The seven horses measured twice at 8 weeks interval were 3-4 year old horses and unbroken that were starting a training program at Holar University College during the autumn of 2003. These horses come from north, south and west of Iceland. During the 8 week period, horses which had GSH-Px-activity 140 U/gr Hb or lower in the first measurement were given an intra muscular injection of 10-20 ml of Selen-E-vitamin and all the horses received a daily ration of 70 g Racing Mineral, which contains 10 mg natriumselenit/kg DM (0,7 mg Se/day/horse).

Glutathione peroxidase activity in whole blood was determined with a spectrophotometric assay originally described by Pagila and Valentine (1967), using Ransel Kit obtained from Randox Laboratories Ltd, UK. The results are expressed as units $grHb^{-1}$.

## Results

The GSH-Px activity in blood from Icelandic horses (n=77) was 139 ±72 U/gr Hb (mean±sd), ranging from 41 to 365 U/gr Hb (Table 1).

Table 1. Blood samples categorised according to GSH-Px -activitiy (n = 77)

| GSH-Px (U/gr Hb) | Number of Samples | Percent % |
|---|---|---|
| 0-50 | 6 | 7,8 |
| 51-100 | 22 | 28,6 |
| 101-150 | 17 | 22,1 |
| 151-200 | 14 | 18,2 |
| 201-250 | 15 | 19,5 |
| > 250 | 3 | 3,9 |

The GSH-Px activity increased by 60% in the blood of the young horses that received intramuscular injection of selen-E and/or Selen-E food supplement (Table 2).

Table 2. GSH-Px activities in blood samples from 7 horses measured at the beginning of a training program and then 8 weeks later. All the horses received a daily ration of 70 g Racing Mineral that contained 10 mg natriumselenit/kg DM (0,7 mg Se/day/horse). In addition, horses that showed GSH-Px activity of 140 U/gr Hb or less in the first measurement (horses nr. 1, 3, 4 and 5 in table 2) were given an intra-muscular injection of 10-20 ml Selen-E-vitamin.

| Horse nr. | GSH-Px activity measured 25.sept.03 | GSH-Px activity Measured 26.nov.03 |
|---|---|---|
| **1** | 67 | 136 |
| **2** | 242 | 365 |
| **3** | 76 | 159 |
| **4** | 64 | 227 |
| **5** | 140 | 188 |
| **6** | 228 | 283 |
| **7** | 229 | 313 |
| **Average** | 149 | 239* |
| **S.D.** | 82 | 85 |
| **Min** | 64 | 136 |
| **Max** | 242 | 365 |

*The GSH-Px activity increased significantly (p<0,001) between measurements (paired t-test).

## Discussion

The results of this study show that there is a considerable variance in blood GSH-Px activity in Icelandic horses. However, it should be kept in mind that the samples came from a group of horses that in some cases were suspected of showing symptoms of selenium deficiency.

Therefore, these measurements can likely not be regarded as representative for Icelandic horses. In 35,4% of the samples GSH-Px activitiy was ≤ 100 U/gr Hb which is used as a marginal value to indicate selenium deficiency at the Institute Experimental Pathology, University of Iceland. Horses in training need more antioxidants e.g. selenium and E-vitamin for muscular function than idle horses (Lewis, 1995). Moreover, horses in training in Iceland are primarily fed on hay that likely contains low levels of selenium, during the main training season from December to June. At present, there is a paucity of information on the selenium status of Icelandic horses and further studies are required, in particular for horses in training.

The GSH-Px activity increased by 60% in the horses that were either only fed mineral supplement or received a supplement and an injection of selenium. At the end of the treatment three of the horses showed GSH-Px activity higher than 250 U/gr Hb. Selenium can be toxic for horses (Lewis, 1995), and therefore it is also important to establish the maximum acceptable values of GSH-Px activity in Icelandic horses.

It can be concluded from this study that; 1) there is a wide range of GSH-Px activity among Icelandic horses 2) GSH-Px activity in blood is increased by injecting selen-E and through regular feeding of selenium 3) further research is required to identify the critical values for GSH-Px activity in Icelandic horses

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# Is molybdenum limiting for nitrogen fixation in clover in Icelandic bog soil?

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## Introduction

Molybdenum (Mo) is necessary for the enzymes that reduce nitrate in plants and for the enzyme nitrogenase which is the key enzyme in biological nitrogen fixation. Because of the symbiotic nitrogen fixation of the legumes, they need higher amounts of Mo than most other plants. Yet the amount needed by legumes is very little and most soils contain adequate molybdenum, in form of molybdate ion, for most plant species. In soils with low pH however molybdate becomes less available and deficiency may occur in plants which need rather high amount of molybdenum.

Mo content in most soils in the world lies in the interval 0.2 – 5.0 mg/kg. In basalt rock the average Mo content is 1.0 mg/kg (Guðni Þorvaldsson et al., 2003). Icelandic soils are derived from basalt and volcanic materials with rather high pH, therefore severe Mo-deficiency is not likely. In peat soils the pH can be low and there is a possibility that biological nitrogen fixation by legume-rhizobia symbiosis could be limited by low available molybdenum. There are not many results from soil analyses were Mo content of Icelandic soils has been measured. Some results from eastern Iceland show unfertilized soil to contain 0.5 – 0.8 mg/kg (Guðni Þorvaldsson et al., 2003). It has been found that the molybdenum concentration in Icelandic rivers and lakes is very variable and in some areas very low and might in some cases be limiting for primary productivity in some lakes (Sigurður Reynir Gíslason, 2004).

It has been shown that different strains of rhizobia differ in their ability to import Mo in the cells and can respond differently to the addition of Mo to plant growth medium. Acetylene reduction has been shown to increase most in symbiotic systems, after addition of molybdenum, when plants were inoculated with strains that were poor at importing Mo (Graham, L. & Maier, R.J., 1987).

The amount of Mo needed to correct deficiency in fields is estimated 70-250 g/ha. This correction can be done by soil and foliar application and by seed treatment. Most often molybdenum is applied as sodium molybdate or ammonium molybdate (Martens, D.C. & Westermann, D.T., 1991). The effect of a single application may last for 5 or 6 years (Russel, E.W, 1973). This is often a cheaper method than application of lime.

About half of the cultivated agricultural land in Iceland is on drained bogs or mires. In southern and western Iceland many farms are dependent on bogs for their hay production. The pH of such soils is typically between 4 and 5.5, highest were mineral content is high. If legumes are to be important crops in Icelandic agriculture, it is necessary to know the factors that could limit their growth in drained bog soil. One of the limiting factors could be low level of available molybdenum in the soil.

The experiment which is described here was a part of a field test of *Rhizobium* strains, and was intended to provide more knowledge about possible role of Mo in reducing yield of clover in bog soil.

## Material and methods

The experiment was established on a dried bog in a farm in South Iceland in the spring 2002. Both red and white clover in mixture with grass were inoculated with five *Rhizobium*-strains in five replications. Inoculation was done by watering the plots with bacterial suspension when most of the plants had got the first leaf in 2002. Control plots were not inoculated. Two of the strains were strains which are sold to farmers for inoculation of clovers, one from Finland (PL) and the other from Sweden (HL). Strain 3 was isolated from white clover nodules in Iceland and D is from DSMZ in Germany. The fifth strain (M) is isolated from white clover in northern Norway. Two of the replications were fertilized with sodium molybdate. The molybdate was applied to the plots by watering young plants with water solution of sodium molybdate equivalent to 111 g Mo/ha. This was repeated in the spring 2003. The experiment was harvested on July 7th in 2003 and July 14th 2004. The yield was divided into grass and clover portions.

## Results and discussion

The Mo treatment had no significant effect on the grass yield. The dry matter yield of clover is presented here (table 1 and 2). The yield of the clover species was low, mainly because of competition from the grasses.

Table 1. Yield of white clover in $g/m^2$.

| | 2003 | | 2004 | |
|---|---|---|---|---|
| *Strain* | *No Mo* | *Mo* | *No Mo* | *Mo* |
| Control | 4 | 7 | 1 | 0 |
| Str. 3 (Icel.) | 7 | 1 | 1 | 5 |
| D | 3 | 9 | 1 | 2 |
| M | 9 | 20 | 7 | 1 |
| HL | 6 | 21 | 2 | 18 |
| PL | 9 | 0 | 5 | 0 |
| **Mean** | **6** | **10** | **3** | **4** |
| St. dev. | 8.1 | 9.9 | 4.2 | 9.9 |

Table 2. Yield of red clover in $g/m^2$.

| | 2003 | | 2004 | |
|---|---|---|---|---|
| *Strain* | *No Mo* | *Mo* | *No Mo* | *Mo* |
| Control | 13 | 12 | 43 | 16 |
| Str. 3 (Icel.) | 31 | 13 | 73 | 57 |
| D | 56 | 56 | 192 | 86 |
| M | 55 | 86 | 147 | 87 |
| HL | 48 | 35 | 117 | 57 |
| PL | 68 | 77 | 235 | 112 |
| **Mean** | **45** | **47** | **135** | **69** |
| St. dev. | 19.8 | 24.2 | 124.8 | 47.3 |

White clover benefited more from the Mo fertilization than red clover. Certain strains (M and HL) responded more effectively to Mo than the other strains. Mo treatment did not increase the yield of red clover except in plots inoculated with the M strain in the first year.

Despite the fact that Mo fertilization did not increase the yield significantly there is a significant difference between treatments with different strains. This experiment is a part of series of field tests of strains which has been going on since 1999 (Halldór Sverrisson & Sigríður Dalmannsdóttir, 2005). The results show that nitrogen fixation as estimated by dry matter yield is different with the different strains used for inoculation. Also the strains differ in their efficiency between the two clover species. The strain M has often given the best result on white clover but has not been high yielding on red clover. PL on the other hand has been very effective on red clover but not on the other clover species.

This is the first time Mo fertilizer has been tested on clover in Iceland. Because of the low yield percentage of clover, especially white clover, the standard deviations are reasonably high. Further investigations on the Mo situation in Icelandic soils are needed.

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# Essential trace elements in the diet of Icelanders
# The Icelandic National Nutrition Survey 2002

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**Aims**
The aim of the National Nutrition Survey was to assess dietary habits, nutrient intake and lifestyle factors in Iceland.

**Methods**
The Icelandic National Nutrition Survey was carried out in 2002. 1242 individuals, randomly selected from the National Register, aged 15-80, participated in the survey; the net participation rate was 70.6%. The dietary assessment method was a 24-hour recall interview by telephone. For quantification, participants received a booklet with pictures of 4 portion sizes of 49 dishes of food, in addition to common household measures. All data were entered directly into an interview-based program, ICEFOOD, developed for this study. 452 food codes from the Icelandic Nutrition Council recipe database were used to enter and calculate the consumption data. These, in turn, were based on 394 items from the National Nutrition Database, ISGEM. Intake of 138 nutrients was calculated, including the trace minerals iron, zinc, copper, iodine and selenium. Nutrient losses due to food preparation were taken into account in the calculations.

**Results**
The mean intake of zinc, copper and selenium exceeds recommendations in all age groups of men and women, while iron and iodine intake is below recommendations among women. The mean iron intake is around 80% of recommended intake in women, aged 15-39 and 60-80 but 62% in those aged 40-59. Iron intake has increased since 1990 mostly because of an increase in the consumption of fortified breakfast cereals. Almost half of dietary iron is derived from cereal products, mostly fortified breakfast cereals (26%) and bread (12%). Meat and meat products contribute 20% to iron consumption.

Iodine intake has decreased since 1990, especially among young girls (15-19 years old) who now get 2/3 of the recommended intake, while the intake was well above recommendations in 1990. The main reason for the reduction is lower fish consumption. High fish consumption has traditionally been one of the characteristic features of the Icelandic diet, but since 1990 fish consumption has decreased by 30%, the greatest decrease being among young people, who now eat almost five times as much pizza as fish. Despite lower fish consumption, fish and fish products contribute most of the iodine in the diet, or 41%, while 36% is derived from dairy and cheese and 5% from cereal products.

**Conclusion**
The mean intake of the trace minerals zinc, copper and selenium exceeds recommendations, while iron and iodine intake is below recommendations among women. Almost half of the iron intake is derived from cereal products, including fortified products. Lower fish consumption has resulted in decreased iodine intake, especially among young girls. Action clearly needs to be taken to reverse this trend. However, iodine fortification possibly has to be considered in Iceland to ensure iodine sufficiency.

# Copper, zinc and manganese uptake and interactions in barley in a field trial

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## Introduction

Copper (Cu), zinc (Zn) and manganese (Mn) are essential micronutrients, which plant takes, at most, a few kilograms per hectar. In acid soils of Finland, deficiencies of these micronutrients are seldom observed. However, uptakes of these nutrients may have antagonistic effects, e.g. Cu has shown to depress Zn uptake (Beckett and Davis, 1978) and Zn has decreased Mn uptake (Graham et al., 1987) of barley. These experiments were conducted in nutrient solution and plant materials were harvested in vegetative growth stage. Therefore the results may be different in field grown crops or the concentrations of nutrients may differ in harvested (grain) products.

A field experiment was conducted to evaluate the effectiveness of $CuSO_4$, $ZnSO_4$ and $MnSO_4$ as fertilizers, with barley as a test crop. Uptake and interaction of these nutrients with each other were evaluated and their distribution between grain and straw.

## Material and methods

Barley (*Hordeum vulgare*) was grown on a clay soil (pH 6.3), with organic matter content of 5.2%. Ammonium acetate- EDTA (AAAc-EDTA, pH 4.65) extractable Cu, Zn and Mn concentrations in the soil were 6.0, 1.6 and 48.6 mg $l^{-1}$ soil. According to Finnish fertilizer recommendations these values are good, low and satisfactory, respectively. Sowing was done with a combined drill and Cu, Zn and Mn were placed with NPK fertilizer (80, 34, 66 kg $ha^{-1}$) to a depth of 8 cm between every second barley row. Following amount of Cu, Zn and Mn were applied: 5, 10, 20 and 40 kg $ha^{-1}$ of Cu, 4.6, 9.2, 18.4 and 36.8 kg $ha^{-1}$ of Zn and 9.4, 18.8, 37.5 and 75 kg $ha^{-1}$ of Mn. They were applied as sulphates. Control plots did not receive Cu, Zn or Mn. The size of the plots was 10*1.5 m.

After harvest, the concentrations of Cu, Zn and Mn in barley seeds and straw were analyzed after dry ashing (Anon 1986). Soil samples were taken in autumn after the harvest to a depth of 20 cm, directly from the fertilizer rows (ten subsamples). Soluble Cu, Zn and Mn concentration in the soil samples were extracted with AAAc-EDTA (pH 4.65).

## Results and discussion

Barley yields (2784 - 4959 kg $ha^{-1}$, 3909 kg $ha^{-1}$ in the control treatment) were not affected by the fertilizer treatments, indicating that soil reserves of these nutrients were adequate for barley growth. Cu application did not affect Cu concentration of barley grain or straw (Figure 1). However, Cu concentration and barley yield correlated positively ($R^2$ = 0.80), although Cu concentration in barley grains was above the deficiency concentration range (Marschner 1995). Application of both Zn (Figure 2) and Mn (Figure 3) increased grain and straw concentrations, the increase being more pronounced in straw. The concentrations of Zn and Mn in the control treatments were above the deficiency concentration range (Marschner, 1995), indicating luxury uptake of these nutrients after fertilizer application.

Total uptakes of Cu and Zn were closely correlated ($R^2$= 0.95) after Cu application. Also Cu and Zn concentrations were correlated in grain ($R^2$ = 0.83) and straw ($R^2$ = 0.90), whereas Cu and Zn interaction was weaker after Zn fertilization ($R^2$ = 0.73, uptake and $R^2$ = 0.20, concentration). Similar results with barley shoots were obtained by Luo and Rimmer (1995). They suggested that Cu application increased Zn concentration in the soil solution, and that the increased Zn uptake was not due to any mechanism within the plant. In the present study

Cu application did not increase AAAc-EDTA extractable Zn from the soil (Table 1), indicating that the increased Zn uptake was a plant-derived process. This hypothesis is supported by the correlation of Cu and Zn uptakes ($R^2$ = 0.97) and the concentrations ($R^2$ = 0.78) in Mn treatments, which did not receive Cu or Zn fertilizer. Concentrations of Cu and Zn did not correlate with Mn concentration after any of the fertilizations.

These results show that in acid soils, Mn and especially Zn concentrations in barley grain can be increased with fertilization. Cu concentration and uptake is probably much more difficult to control. Cu and Zn uptake seems to interact positively and Cu application had more intense effect on the uptake of these nutrients.

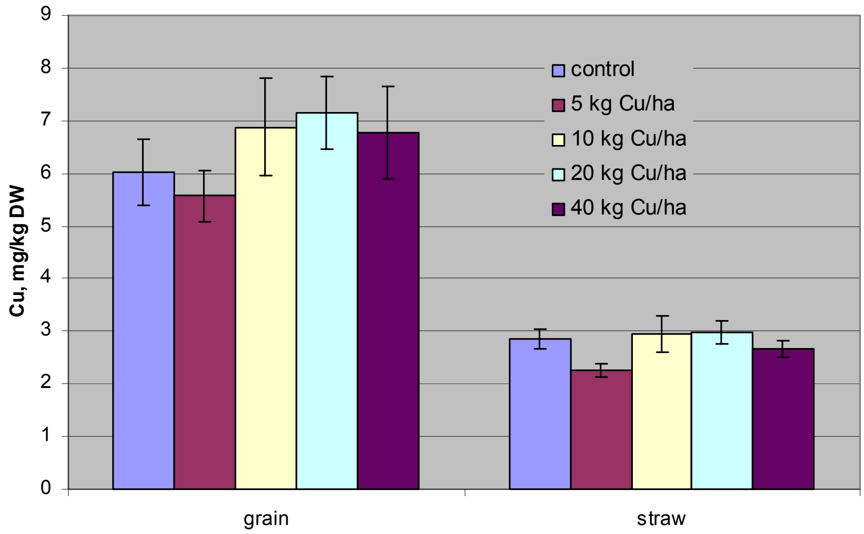


Figure 1. Cu concentration in barley grain and straw at different levels of Cu application.

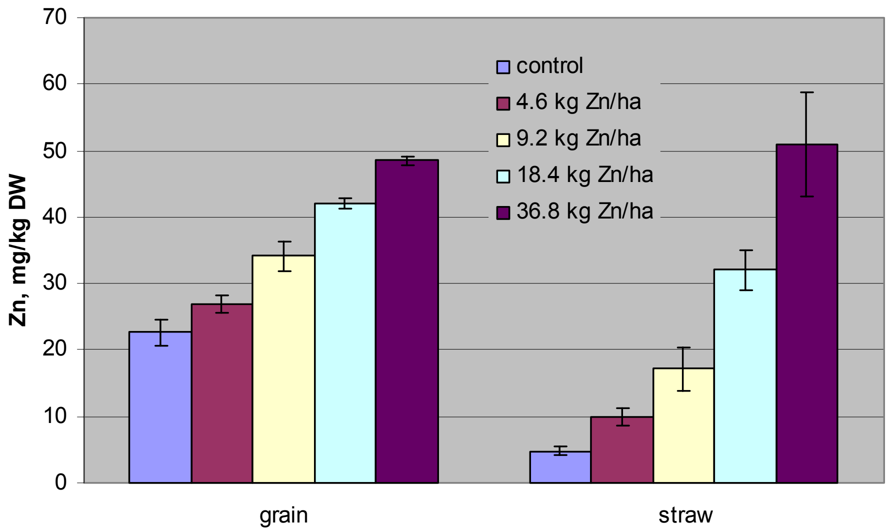


Figure 2. Zn concentration in barley grain and straw at different levels of Zn application.

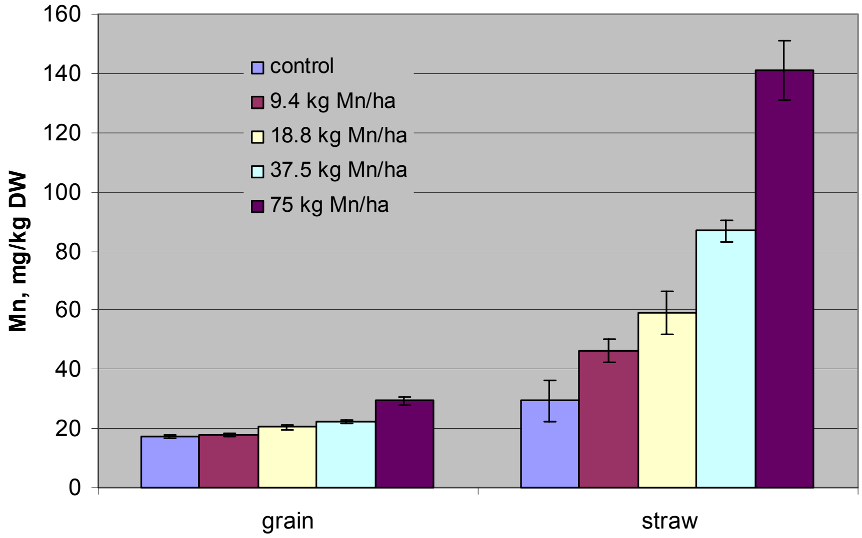


Figure 3. Mn concentration in barley grain and straw at different levels of Mn application.

Table 1. Cu, Zn and Mn concentrations (AAAc-EDTA, mg $l^{-1}$) in soil samples, which were taken at the points of the fertilizer rows.

| Treatment | Cu | Zn | Mn |
|---|---|---|---|
| 5 kg Cu $ha^{-1}$ | 8.8 | 1.6 | 34.0 |
| 10 kg Cu $ha^{-1}$ | 9.8 | 1.4 | 32.7 |
| 20 kg Cu $ha^{-1}$ | 15.8 | 1.5 | 32.5 |
| 40 kg Cu $ha^{-1}$ | 25.4 | 1.5 | 30.4 |
| 4.6 kg Zn $ha^{-1}$ | 6.3 | 5.4 | 32.2 |
| 9.2 kg Zn $ha^{-1}$ | 5.5 | 9.9 | 34.6 |
| 18.4 kg Zn $ha^{-1}$ | 5.4 | 14.9 | 34.2 |
| 36.8 kg Zn $ha^{-1}$ | 5.8 | 33.9 | 30.8 |
| 9.4 kg Mn $ha^{-1}$ | 6.4 | 1.7 | 51.9 |
| 18.8 kg Mn $ha^{-1}$ | 6.2 | 1.6 | 53.8 |
| 37.5 kg Mn $ha^{-1}$ | 5.6 | 1.5 | 75.1 |
| 75 kg Mn $ha^{-1}$ | 5.8 | 1.6 | 83.8 |

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