Pure Appl. Chem., Vol. 78, No. 1, pp. 111–133, 2006.
doi:10.1351/pac200678010111

Selenium in agriculture, food, and nutrition*

M. Sager

Austrian Agency for Health and Food Safety, Competence Centre for Elements, Spargelfeldstrasse 191, A-1226 Vienna, Austria

Abstract: In the case of Se, the concentration range between essentiality and toxicity for terrestric animals and humans is rather narrow, while aquatic organisms are much less affected, and no essentiality to green plants and aquatic macrophytes has been established yet. This review focuses on the situation in Europe, where Se levels are generally low. Apart from industrial and mining activities, the main Se sources are the burning of coal and selenite additions to animal feedstuffs. Reduction processes in sediments, soils, and feedstuffs to yield elemental Se act as sinks for available Se forms. In soils and crops, Se levels get enhanced from recycling of manure, dung, and sewage sludge, which is beneficial for Europe. New data from Austria have been added to the detailed discussions. In human nutrition, Se is supplied via pork, liver and kidneys, seafood, and cereals, but main sources as well as blood Se levels vary between different countries and nutritional habits. Food processing, like boiling, baking, or grilling, results in Se losses.

Keywords: selenium; selenium supplementation; metabolic pathways; trace analytical methods; food.

INTRODUCTION

Selenium was discovered by Berzelius as early as in 1817, but it needed a further 140 years until its nutritional essentiality was proved in 1957. This led to a tremendous need of data, development of analytical methods, and biological studies. High- and low-Se areas were classified worldwide, finally, there has been an attempt to raise low-Se nutritional levels by large-scale addition of sodium selenate to fertilizers in Finland since 1984. Toxicity and essentiality have been widely discussed by many authors [1,2], and within the last 10 years, some 100 000 references referring to the keyword "selenium" have been published.

Whereas in Europe, the main problem is how to ensure sufficient Se supply in human nutrition, intense work has been done in India and the United States to remediate seleniferous sites and ground waters in order to avoid Se intoxications [3]. Even transgenic plants have been designed for phytoremediation of seleniferous sites [4], which is forbidden in Europe, and therefore beyond the scope of this paper.

DETERMINATION OF TOTAL SELENIUM CONTENTS

It may be beyond the scope of this article to give a general review of analytical methods. Within the last decade [2,5], inductively coupled plasma–mass spectrometry (ICP–MS) has appeared as a major inno-

*Paper based on a presentation at the 2nd International Symposium on Trace Elements in Food (TEF-2), Brussels, Belgium, 7–8 October 2004. Other presentations are published in this issue, pp. 65–143.

vation for total element as well as isotope analysis, including Se. Its huge sensitivity has opened the scope for the discrimination of Se-containing molecules as a sensitive detection tool after sophisticated separations. For routine applications in smaller labs, hydride methods and graphite furnace atomic absorption spectroscopy (AAS) have remained state-of-the-art methods, whereas spectrophotometry and fluorimetry, electrochemical methods, and nuclear techniques are disappearing.

Final determination by hydride atomic absorption or extraction–spectrophotometry, respectively, extraction fluorimetric methods require conversion of any Se species into selenite, which might be done with hydrochloric acid at the boiling water bath. Electrochemical methods are also species-dependent. In ICP–MS, calibration should be done with the same species, as ionization efficiency of different compounds might be variable [6–8]. Addition of a small amount of methane (10 ml/min) into the coolant gas channel improved the ionization of Ge, As, and Se, and increased the analytical sensitivity at least two-fold [9]. ICP–optical emission spectroscopy (OES) is very rugged to Se speciation, but not sensitive enough for environmental levels. In graphite furnace AAS, any Se compound has to be thermally stabilized by charring in the presence of suitable cations (e.g., Pd), due to volatilization losses [10–14].

During drying of wet samples, like sludges, manure, or sediments, or even Se accumulator plants, Se might be partially volatile as hydrogen selenide or its alkylated derivatives [15]. Sample drying has to be done under oxidizing conditions (e.g., in the presence of excess nitrate), unless the wet samples are digested as such. The validity of the sample drying procedure cannot be proved using certified reference materials, as they had been already dried before the certification campaign.

In order to avoid volatilization of Se during the sample digestion, oxidation with nitric acid in closed systems (for small sample weights of foods) [16], with aqua regia under reflux (for minerals, especially sulfidic ores) [17,18], or ashing with magnesium nitrate [19,20] (for feedstuffs, sludges, and manure) have been used in my laboratory. Liquid samples can be charred directly in the graphite furnace in the presence of suitable cations (e.g., Pd) prior to AAS determination.

METHODS FOR INVESTIGATING SELENIUM SPECIATION

A thorough review of this rapidly growing scientific field would be beyond the scope of this article. The subsequent short summary should explain to the reader why so many details about Se cycling and metabolism are known today, and which tools are currently available to perform further studies.

Speciation studies may aim either at Se-containing molecules, seleno-amino acids after destruction of the proteins, or the molecular weight of seleniferous proteins, by suitable separation and element specific detection. The mobile phase has to match the requirements of the detection system. Prior to speciation studies in solution, it is essential to dissolve solids, or leach them, respectively, to extract all seleniferous compounds, while keeping their structure intact.

For separations of Se-containing species in liquids, high-performance liquid chromatography (HPLC) at anion exchange columns [8,21,22], reversed-phase columns [22–25], or size exclusion columns [22,26–28], have been successfully coupled with element sensitive detectors, such as ICP–MS. This simplifies the resultant chromatograms. The detector system limits the application of organic solvents.

Information about Se speciation in tissues may be achieved after enzymatic degradation of proteins or carbohydrates, to yield Se-containing amino acids or maybe other metabolites [8,24]. Macromolecules may be chromatographed after minor preparations [27]. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate permits the direct separation of Se-containing proteins in subcellular fractions of, for example, liver and plasma samples [29,30]. Information about speciations in environmental solids, like soils, sediments, sludges, and manure, can be obtained from selective leaching procedures, which, however, have to be adapted to the special chemical properties of Se. Sequences developed for cationic elements are not suitable. Selenate, selenite, organically bound Se, sulfidic bound Se, and elemental Se should be discriminated, as they are different in mobility as well as in plant availability (see below).

Speciations in the gas phase have been investigated after suitable stripping, trapping, and desorption by various gas chromatographic methods [21,31,32]. Element-specific detection systems are preferable, but conventional detectors have also been used. Some seleniferous gaseous compounds are sensitive toward oxidation by oxygen. Due to thermal instability, maximum temperature of gas chromatography (GC) is limited to about 140 °C.

SELENIUM IN THE ENVIRONMENT

Natural waters

The Se content of natural waters can vary within a broad range, from <2 ng/l up to 300 µg/l. High Se levels can be expected under alkaline, oxidizing and low organic carbon conditions, and also in eluates from sulfidic deposits as well as close to volcanoes [33–37]. In Europe, contaminations may mainly derive from manure and dung of Se-supplemented animals, from sewage sludges, from burning fossil fuels and respective ashes, or from selenate-containing fertilizers [38].

In oxic waters, homogenous oxidation of selenite to selenate by dissolved oxygen does not occur [39]. In suboxic and anoxic zones of deep-water bodies, selenide and organoselenium compounds like amino acids, but no dimethylselenide, have been found. Under natural nonsterile conditions, soluble organoselenium compounds are not stable, presumably because of efficient active uptake by bacteria [40].

Selenium is incorporated into many biota in the surface waters, and liberated in deeper layers, when dead biota or fecals dissolve. Free selenide is precipitated with pyrite, or as ferroselite $FeSe_2$ [31]. For use as potable water, the upper tolerance limit in Austria is 10 µg/l (Austrian Federal Law: BGBl 1995/359), which is hardly reached in Europe.

Total content in soils and sediments

In Europe, Se content in arable soils is usually met within a narrow and rather low range. Parts of the United States, Australia, and India are significantly higher in Se, and have led to concerns and remediation strategies, which are beyond the scope of this paper (see [3,4]). Within the Austrian soil-monitoring program, median values of total Se for various soil types ranged between 0.11–0.41 mg/kg for total Se, irrespective of the geological facies below [41].

At least in Austria, the impact of soil use is much larger than the influence of the geological facies met below, like fertilization practices, or transfer to crop plants, whereas washout to deeper soil layers is low. Soils from corn production areas were significantly lower in Se (0.20 mg/kg) than soils in the greenland (0.29 mg/kg) [41]. Chernozem soils at a fertile and flat but very remote area (near Parndorf, Austria), where there has been no settlement for 1600 years, had a median of 0.23 mg/kg Se, and Se in subsoils was not significantly different [44].

In Finland, a mean total Se content of 0.21 mg/kg in the plough layer was found, highest levels were met in organogenic soils [38]. In Norway, levels of Se in soils have risen from a mean of about about 0.2–1.4 mg/kg in high-precipitation areas in the west of the country [42].

According to Austrian standard ÖNORM L1075, arable soils containing more than 1 mg/kg Se are suspicious to contamination, and soils, respectively, garden molds, containing more than 5 mg/kg should not be used for agricultural puposes. In China, where large regional differences in soil-Se occur, the soils of a Se content 0.09–0.17 mg/kg are classified as Se deficiency regions, and more than 1.70 mg/kg Se as toxicity regions [42]. In sediments in the United States, the predicted level of toxic effects is set at 2.5 mg/kg, and the observed level of toxic effects is set at 4.0 mg/kg [43], but adaptations of local wildlife toward higher ambient Se levels are possible.

Selenium in coals

Se in brown coal sampled in Austrian power plants ranged from 0.80–19.4 mg/kg, and hard coal from 0.29–5.6 mg/kg [50]. Further data have been compiled in [44]. Corresponding slag and fly ash samples were within the same range, whereas significant Se enrichments have been reported from Great Britain, 2.1–23.2 mg/kg [51] and 4.1–440 mg/kg [46].

The recovery of Se either in bottom ash or in fly ash greatly depends on the combustion process and the alkalinity of the bottom slag. In coal combustion, the trace elements associated with the organic matter and in organic sulfides like Se volatilize more readily than elements present in aluminosilicate minerals, and preferably move to fly ash and finer grains. If the slag-bottom ash is alkaline, however, its Se content is high. Volatiles are likely to condense on the surface of the particles [45–49].

Estimation of selenium speciation in soils and sediments by selective extractions

Due to special chemical properties of Se, its mobility can hardly be estimated from similar mobile fractions established for cationic "heavy metals", thus, a proper sequence has to be established. Selenate, selenite, elementary, sulfidic, and organically bound Se should be discriminated. There are no carbonates to consider, and starting with reduction might precipitate elementary Se and thus cause artefacts. In alkaline, highly oxidized soils (pH 7.5–8.5) Se(VI) has been reported as the dominant soluble forms of Se. Selenium was mostly adsorbed as Se(IV), but readily oxidized to soluble selenate ions, which are highly available to plants [52]. Seleniferous soils from Ireland contained 2 % of total Se as water-extractable selenate [53].

Extraction of soils and sediments with neutral phosphate or citrate/phosphate buffer solutions yields an exchangeable fraction, which can be investigated for further speciation in solution [54–58]. After removal of solubles and exchangeables, oxalate pH 3 selectively extracts selenite bound to iron hydroxides, which is the main fraction in acid soils. The extract can be directly used for hydride-AAS determinations, but the stability is moderate (a few days) [59,60]. The Fe/Mn oxides should be dissolved by 4 M HCl, but not pyrite [54].

There are good chances to dissolve the organics with an alkaline extractant like the classical separation of humics, leaving the selenides untouched, but some Se-bound organic materials may be found already in the water extract. Only >1 % of elemental and <2 % of metal selenide was solubilized by 0.1 M NaOH extraction, indicating its high selectivity for organically associated Se [61]. In sewage sludges, which are low in residual minerals and extremely high in organic carbon, total Se could be found in the $NaOH^-$ extract [56]. In wetlands, organic forms of Se are subject to greater bioavailability and bioaccumulate more rapidly than selenite and selenate [60]. In this case, the structure of the extracted organic matter is also investigated, oxidation has been prevented by use of 0.1 M NaOH/0.1 M $Na_4P_2O_7$ extract, flushed with N_2 [62,63].

Elemental Se dissolves in neutral and alkaline sulfite solutions (pH >7) to yield $SeSO_3^{2-}$, within 1 h at room temperature, or 10 min boiling [60,64]. Residual Se bound to sulfides and selenides may be finally obtained from aqua regia digestion.

Speciation and mobility in soils and sediments

Vertical mobility in soil columns greatly depends on the speciation of Se, soil physical–chemical factors such as redox behavior, pH, or microbiological activity [2,59,65]. Whereas selenate is rapidly leached from soil columns, it is microbially reduced in soils to selenite and organo-selenium compounds, and to a minor part volatilized via methylation [66], depending on the organic carbon contents and the oxygen in the pore gas.

Sorption of selenite by soil showed some analogs with the sorption of phosphate, whereas sorption of selenate was closer to sulfate, with respect to activation energy, binding constant, and diffusion

term [67]. In soil columns, selenite was largely fixed from mineral fertilizer solution, and constantly released by daily addition of water to deeper layers as non-selenite Se. Like for sulfur, drying and aeration periods enhanced Se mineralization, thus increasing its mobility [59].

Under reducing conditions, Se may be present also in elemental form, which is available just for certain bacteria.

Soil-to-plant transfer

Amino acids, selenite, and selenate can pass directly into plant roots. Under neutral, oxidizing, and low-carbon conditions, Se uptake is governed by drainage water rather than concentration in the soil [68], and water-soluble Se of soils correlated better with the uptake of Se by plants [69]. Green plants (vegetables) take Na-selenate from soil solution more readily than selenite [15], which is why seleniferous mineral fertilizers contain Se as selenate.

In Finland, hot-water-extractable Se was used as an indicator of plant availability, which ranged between 1.5–10.2 % of total (mean 4 %) [38]. Organic Se from Se-accumulating plant material and inorganic Se were readily available for uptake by many plants. Plants have been shown to actively absorb several amino acids, like methionine, and presumably Se-methionine [70]. The tendency of plants to accumulate Se partially corresponds with their sulfur requirement, but competition between sulfate and selenate can also reduce selenate uptake. Leaves and kernels usually have higher Se contents than roots and stalks [70].

Elementary Se is highly inert. Humic-bound Se will be of increased availability after destruction of the organic material. In acid soils (pH 4.5–6.5), selenite is usually bound to iron hydroxides, which is commonly regarded as unavailable to plants.

Within a project performed by the author [71], wheat and maize samples harvested at three locations in lower Austria were as low as 4–10 μg/kg Se. In order to raise the Se uptake rate via nutrition for humans, the fertilization for various cereals was done utilizing a 20:8:8 mineral fertilizer with 16 mg/kg Se as selenate (like in Finland), because selenate is supposed to be taken up from soil by plants up to 10 times more effectively than selenite [72,73]. There was linear uptake of Se, and transfer to maize grains was lowest. In the field, plants grown at the cambisol had the highest Se concentrations, and at the high adsorptive clay soil, the lowest, though the mobility of anions should be higher at higher pH. Marked differences between pot and respective field experiments appeared. Increase of Se concentration from the pots was higher, and Se preferably moved to the straw. In the fields, the Se utilization rate was about 2 % of added Se, and some memory remained for the subsequent year [71]. The Se in the plants was found largely metabolized as selonomethionine [8]. Similarly, in Finland, Se levels in cereals have been raised in general by selenate-containing mineral fertilization [38].

Some higher plants and mushrooms are known to accumulate Se up to 100 mg/kg, for example, *Astralagus* or *Boletus edulis* [74]. Garlic grown on seleniferous soil in China contained 205 mg/kg dry weight (dw) [24], and *Astralagus bisulcatus* collected in the Shirley Basin near Medicine Bow, Wyoming, contained even 300 mg/kg dw [75]. One of the accumulation mechanisms for Se-tolerant plants is the formation of organoselenium compounds that cannot be incorporated into proteins, thereby avoiding toxicity. *Brassica juncea* (Indian mustard) accumulated Se when grown hydroponically in the presence of selenite, selenate, and selonomethionine, forming Se-methylselenocysteine, a non-protein amino acid [76]. Apart from accumulation at seleniferous soils, high Se levels in green plants might be expected at landfill sites containing disposed fly ash. In the United States, where 75–80 % of the fly ash is disposed of in landfills, these landfill sites are capped with about 60 cm soil and recultivated. The amount of accumulation largely depended on the crop species, the part of the plant, and the growing season; it can even reach levels toxic to grazing animals. Plants with a high requirement for sulfur (Brassicaceae) also took much Se. Sulfur, applied as gypsum, reduced Se uptake in alfalfa and oats because of competition, but was of no effect in other crops [70].

Aquatic ecosystems

Se has not yet been proved to be essential for phytoplankton or aquatic macrophytes [77], and their Se content may vary widely according to environmental levels as well as competitive sulfate and phosphate levels. The growth of bacterioplankton from an oligotrophic lake was found to be stimulated even at 0.55 µg/l selenite-Se [78]. Blue–green algae are more tolerant toward Se than green algae and diatoms. Thus, the cyanobacterium *Anabaena flos aquae* easily survived 10 mg/l selenate for 10 days [79], and S*cenedesmus dimorphus* and *Anabaena cylindrica* showed just reduced growth and reduced phosphate uptake at 40 and 80 mg/l levels of selenite and selenate-Se [80]. In the green alga *Chlamydomonas reinhardtii*, the uptake of soluble selenate was observed to be linear vs. time, and competitive with sulfate, whereas uptake from selenite was initially faster, but reached a plateau after 4–6 h [81,82].

Submerged aquatic macrophytes *Potamogeton crispus* and *Ruppia maritima* have been tested for phytoremediation capabilities; they are Se nonaccumulators, but occur rather ubiquitously. When they received seleniferous agricultural drainage waters of 12 µg/l selenate-Se, 2 g/l sulfate, and pH 8, for 10 days, root tissues ranged from 0.50–0.54 mg/kg dw, and shoot tissues from 0.36–0.38 mg/kg dw, which is within the range of terrestrial plants. Selenium was metabolized to selonomethionine, and to a lesser extent to selonomethylcysteine, whereas selonocysteine was not detected. About 0.01 % of added Se was finally found as organic Se in the culture solutions. Addition of 1.6 g/l sulfate-S decreased tissue Se to about one-half [77]. Macrophyte decomposition is typically characterized by an initial rapid leaching phase within the first 24 h, followed by a re-absorption phase as the detrital microbial population increases [83].

Selenium in manure, organic amendments, and sewage sludges

Frequently, farmed animals are fed with Se-containing feedstuffs close to the upper permissible level (see below). Excess Se is excreted, and thus leads to enhanced Se levels in manure and in sewage sludges (Table 1 [84]). Organic amendments thus cover a wide and still unpredictable source of Se enrichment for agricultural soils, and Se speciation in these matrices is still an interesting subject of investigation. Anyway, manure has more Se than agricultural soils, except from "bio"-farming. Possible systematic errors from the drying step have been already discussed.

Table 1 Selenium in organic amendments in Eastern Austria. Manure dried in presence of $Mg(NO_3)_2$- ashed in the muffle furnace at 560 °C.

	Median mg/kg	Range		Reference
Manure after biogas production	0.74	–	1998/99	Sager. unpubl.
Solid manure from pigs	3.09	1.08–7.7	1998/99	Sager. unpubl.
Liquid manure from pigs	1.31	1.16–1.41	1998/99	Sager. unpubl.
Poultry dung	1.33	0.54–1.78	1998/99	Sager. unpubl.
Liquid manure from fattening cattle	0.125	0.12–0.13	1998/99	Sager. unpubl.
Liquid manure from dairy cows	0.825	0.804–0.846	1998/99	Sager. unpubl.
U.S. sewage sludges		0.4–9.6		[84]

Microbial transformations in waters, sediments, and soils

The boundary between Se(VI) and Se(IV) is at an Eh of about 250–285 mV, and between Se(IV) and Se(0) at an Eh of about –10 to –40 mV [85]. Large fractions of insoluble elemental Se often prevail under oxidizing conditions [86].

In natural waters, bacteria exhibit Se uptake and incorporation similar to the phytoplankton, and can additionally volatilize Se [87] via methylation. Bacterioplankton can take up more selenate per unit carbon than larger cells [88]. As early as 1960, *Escherischia coli* was shown to metabolize selenite to selonomethionine and selonocystine bound to bacterial proteins [89]. *Enterobacter cloacae* reduced selenite to elemental Se, inhibited by nitrate, but without being effected by sulfate. Optimum conditions were anaerobic, pH 6.5 and 40 °C; but the process was aerobically possible [90]. Flocculation or re-oxidation may remove elemental Se from the water phase, including both abiotic and microbial processes [85].

In anoxic sediments, selenite and selenate were removed by bacterial reduction to elemental red Se. The bacteria were fed with acetate, and the process was unrelated to sulfate reduction. No volatilization and no reaction in autoclaved samples occurred [91]. On the other hand, bacterial biotransformation is the only way to involve elemental Se to the foodweb, via bacterial consumption by zooplankton, and benthic uptake from ingested sediments [87]. Under anaerobic conditions, H_2Se may also be formed [92].

In soils, microorganisms (like *Aspergillus*, *Candida*, *Cephalosporium*, *Penicillium*, and other fungi and bacteria) can reduce selenite to the volatile species dimethyl selenide, dimethyl diselenide, and dimethyl selenone. Biomethylation occurs at 500, 200, and 0 mV. Fungi and bacteria contribute nearly equally. Therefore, the addition of organic matter to the soil increases the volatilization of Se, promoting the microbial activity. Thus, Se is much more volatile (and also mobile) from peat than from normal soil [93,94]. Trimethylselenonium, which is the major urinary metabolite of Se, and which might occur in liquid manure, was found to be rapidly decomposed (within 8 days) by soil microbes to yield volatile dimethylselenide [95].

Dimethylselenide has been considered to be 500 times less toxic than selenite, therefore, methylation is an effective detoxification mechanism done by fungi, plants, and animals. Alkylated Se compounds have been detected in the atmosphere just above soils and sediments [21]. Formation of volatile compounds can happen from any Se species, it is most rapid with selenite, and slowest with elemental Se [92]. Volatilization of spiked selenite from soil depends on the microbial activity, temperature, moisture, water-soluble Se, and, last not least, on the season of the year, when the soil sample was collected. Soils collected in spring evolved most; from autoclaved soil, no volatilization took place. Volatilization vs. moisture content passed a maximum at 28 %, which was far below saturation for the soil used [96]. Addition of selenate to fine-loamy, calcareous, non-water-saturated soil led to volatilization of Se as dimethylselenide or dimethyldiselenide to only 1.8–4.3 % of input [66].

SELENIUM IN ANIMAL PRODUCTION

Selenium in feedstuffs and levels in healthy farmed animals

In Europe, the basic components of animal feedstuffs like cereals are usually below 0.15 mg/kg Se, except fishmeal (Table 2). In order to promote optimum growth and resistance to various illnesses, Se as sodium selenite is permitted to be added to commercial animal feedstuffs up to a total content of 0.5 mg/kg. Other Se compounds are too expensive to be fed to animals. There is still a tendency to feed more Se to pigs than to cattle, horses, or poultry. In addition to home-made basic diets, supplementary food can be given, which has mean levels between 0.54 and 2.35 mg/kg. In feed-processing industrial plants, some percent of so-called "mineral feed" (mean level 12–32 mg/kg Se) is mixed to low-Se components to provide optimum levels of essential trace elements. In commercial agrochemicals like premixes, Se can reach up to 1000 mg/kg, which is highly toxic when consumed undiluted (Table 2).

Table 2 Selenium in commercial feedstuffs controlled in Austria, 2003/2004.

		Se median	Se mean	Se std.	Cu/Se median	Cu/Se mean	Cu/Se std.	Mn/Se median	Mn/Se mean	Mn/Se std.	Zn/Se median	Zn/Se mean	Zn/Se std.
Piglets	Single	0.38	0.48	0.21	232.7	358.0	284.1	215	225	90	366	565	393
Pigs	Single	0.42	0.43	0.06	49.4	61.3	21.0	191	238	100	300	368	158
Piglets	Supplementary	1.75	2.35	1.78	301.5	327.8	104.9	193	218	67	365	457	248
Sows	Supplementary	1.44	1.54	0.68	74.0	88.0	46.6	135	180	99	230	278	127
Pigs	Supplementary	1.79	2.39	1.87	82.3	122.8	106.4	214	325	247	413	601	472
Bulls	Supplementary	0.63	0.65	0.16	60.5	77.0	28.5	184	201	60	306	385	109
Lact. cows	Supplementary	0.68	0.84	0.48	61.6	71.6	39.6	230	246	131	370	443	192
Horses	Supplementary	0.50	0.77	0.44	68.4	75.0	15.6	245	310	109	535	683	278
Piglets	Mineral	10.45	12.01	6.44	274.2	282.9	121.9	176	203	98	337	390	147
Pigs	Mineral	14.90	16.63	8.16	55.2	56.7	22.8	120	134	64	226	231	92
Sows	Mineral	11.30	13.99	7.32	61.9	59.8	21.3	143	159	69	261	250	91
Calves	Mineral	14.30	13.90	1.82	20.9	42.3	31.1	96	101	17	170	191	51
Bulls	Mineral	27.50	31.01	18.14	35.5	47.0	34.2	95	123	105	227	280	208
Lact. cows	Mineral	27.60	31.86	8.18	31.6	33.8	6.9	87	100	39	198	207	69
Horses	Mineral	14.70	15.58	7.09	44.9	47.0	11.1	122	155	58	259	272	73
Sheep	Mineral	18.80	25.86	16.82	2.1	4.3	3.6	133	172	98	324	420	218
Poultry	Mineral	11.80	16.24	7.84	17.4	29.2	17.9	201	253	99	163	199	85
Hens	Premix	46.5	47.2	5.88	40.8	43.6	9.3	507	549	125	215	243	68
Poultry	Premix	563.0	554.0	354.71	34.9	34.6	11.2	287	310	94	217	211	64

Though Se is added as selenite, reactions with other components during storage may change its speciation to yield more than one-half of a nonextractable fraction, possibly elemental Se [97].

Due to different feedings and feeding requirements, serum Se in healthy subjects was investigated in Switzerland. The Se frequency distributions were slightly skewed to both sides [98]. Median concentrations in cattle (24 μg/l), calves (27 μg/l), and sheep (24 μg/l) were lowest, because they are fed on the naturally occurring grass (which is low in Se in Europe throughout), and because in ruminants, Se uptake is generally lower than in monogastric animals and humans. Selenium gets reduced and lost by the microbial flora in the forestomachs. Thus, median Se levels in goats (50 μg/l) and horses (84 μg/l) were higher. Pigs (median 194 μg/l) and laying hens (median 211 μg/l) had significantly higher serum Se levels, because they were fed commercial Se-supplemented food. Dogs (median 261 μg/l) and cats (median 528 μg/l) were fed commercial food as well, which may contain large amounts of liver and kidneys, as well as marine fish for cats only [98]. Contrary to the data above, pigs fed with a Se-deficient diet of 0.035 mg/kg had just 92 μg/l in their blood plasma, which could be raised to 165 μg/l feeding with feeds of 0.485 mg/kg [99]. In rats, Se in feeds did not simply correlate with the glutathione peroxidase activity in the blood plasma, but reached a constant level between 0.45 and 3 mg/kg, and increased again at higher levels [100].

In humans, mean Se values in serum, respectively blood, may vary from 55 to 185 μg/l, due to local Se levels and nutritional habits (data largely compiled in [101]; see Table 3).

Table 3 Selenium in human serum and whole blood in Europe.

Sample	Location	Mean selenium content	Ref.
Human serum	Northeast Bohemia	55 ± 11 μg/l	[133]
Human serum	Graz, Austria	64 ± 11	[134]
Human serum (20–60 years)	Zürich, Switzerland	90 ± 18	[98]
Human serum (60–100 years)	Zürich, Switzerland	88 ± 26	[98]
Human plasma	Pavia, Italy	80 ± 12	[135]
Whole blood	Pavia, Italy	108 ± 11	[135]
Whole blood	Greenland	185	[101]
Whole blood	N. Ireland	91 ± 16	[101]
Whole blood	Southampton, UK	138 ± 19	[101]
Whole blood (fish-consumers)	Sweden	80	[136]
Whole blood (non-fish-consumers)	Sweden	91	[136]
Whole blood	Denmark	109 ± 27	[101]
Whole blood	Netherlands	133 ± 20	[101]
Whole blood	Bruges, Belgium	129 ± 16	[101]
Whole blood	Namur, Belgium	96 ± 10	[101]
Whole blood	Mainz, Germany	92 ± 18	[101]
Whole blood	Poland	101 ± 22	[101]
Whole blood	Ljubljana, Slovenia	88 ± 27	[101]
Whole blood	Central Italy	77	[101]
Whole blood	Northeast Italy	110	[101]
Whole blood	Vrasta, Bulgaria	40 ± 20	[101]
Whole blood	Athens, Greece	165 ± 33	[137]
Whole blood (benign mamma carcinoma patients)	Vienna, Austria	66 ± 21	*
Whole blood (malign mamma carcinoma patients)	Vienna, Austria	71 ± 17	*

*Sager 1988, unpublished.

Deficiency and toxicity symptoms in farmed animals

Suboptimal nutritional Se has led to muscular dystrophy ("white muscle disease") in pigs, cattle, sheep, and horses, sudden heart failure causing death, loss of appetite, retarded growth, impaired fertility, and disturbances of reproduction, which has been observed in Sweden, a low-Se country [103]. In addition, myopathy, cardiomyopathy, and immune dysfunction occur as symptoms of Se deficiency. In special experiments feeding pigs, a 0.1 mg/kg diet was sufficient to reach a plateau activity for cellular glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase in the thyroid or pituitary glands, but enzyme activities in the liver, heart, and lungs were lower. For full activity of all glutathione peroxidases in all tissues, 0.2 mg/kg Se was needed [104]. In pigs receiving corn/soy-based diets of 0.03–0.34 mg/kg Se, overall growth performance, including daily body wt. gain, feed intake, feed use efficiency, and plasma α-tocopherol concentrations were not affected, but the glutathione peroxidase activities corresponded to dietary Se.

In animal farming, wrong calculations in the production of mixed feeds, as well as feeding Se accumulator plants, may lead to serious Se intoxication. At dietary contents of 15–40 mg/kg Se, pigs developed toxic symptoms such as skin lesions, loss of hair, redness of skin, hind limb ataxia, soft hooves, reduced feed intake, and loss of pain response [105]. In spite of varying intoxication levels, mean plasma Se was maintained at a plateau of about 2.3 μg/l. Whereas in pigs of 9 weeks initial age, no signs of poisoning were observed from corn/soybean meal diets containing 1.95 or 8.2 mg/kg Se for 8 weeks, in cattle, 5–25 ppm Se dramatically reduced reproductive performance. High-level exposure during pregnancy caused fetal death in chickens, mice, pigs, and rats, as well as reduced growth rates and malformations [75].

Sheep appear to be more resistant toward seleniferous soils. Chronic intoxications were investigated in ewes that were fed alfalfa containing 24 ppm Se as Na_2SeO_4, and 29 ppm Se metabolized in *A. bisulcatus*, respectively, for 88 days. Controls received alfalfa with 0.8 ppm Se. There was no significant difference in progesterone or 17β-estradiol profiles between treatment groups and controls, nor in estrous cycle length or estrous behavior. All were fed the same amounts of pellets, and all weights remained constant. Blood Se levels were highest in the group fed Na_2SeO_4 (2.4 mg/kg), followed by *A. bisulcatus* (1.3 mg/kg) and controls (0.45 mg/kg). All lambs appeared normal at birth, and there were no differences in weight [75].

Mice fed diets containing selonomethionine at a level of 20 mg/kg Se, and raised to 30 mg/kg at 3 weeks, showed delayed response to Se toxicity, slower recovery from the toxicity, and higher retention of tissue Se, than those fed with equal amounts of selenite or Se-methylselenocysteine [106]. Sodium selenite administered intraperitoneally (ip) to guinea pigs induced changes in cardiac mitochondria, that is, dose-dependent increase in glycogen, and lesions prevailing at least 2 weeks [107].

One-day-old ducklings died within 1 week of receiving a diet of 80-ppm selenite or selonomethionine Se. At 40 ppm, 25 % mortality occurred after two weeks with Na_2SeO_3 and 15 % mortality after 3 weeks with selonomethionine, whereas 10 and 20 ppm caused no mortality in either form. Se treatment significantly decreased body wt. and food consumption [108].

Selenium uptake and metabolic pathways

Most of Se is taken orally from food. Its metabolism is highly regulated and influenced by speciation, needs, and concomitant trace metals. Many experiments have been done with 75-Se labeled compounds.

Oral administration of a single dose of selenite, selenate, selonomethionine, and Se yeast to rats led to maximum levels after 3–6 h, and a decline from 6–24 h. Intensive absorption took place in the gut in all parts, but not in the stomach, leading to marked accumulation in the liver. In serum, Se levels decreased in the order selenate > selenite> selonomethionine> seleno yeast. Selenium from selonomethionine was excreted slower, and had marked higher affinity toward the brain [27,109,110]. In cows,

feeding selenized yeast was 2–3 times more effective in raising the blood Se, liver Se, and glutathione peroxidase activity with respect to soluble selenate, administered via potable water [111]. In grower and finisher swine, Se retention was also higher from selenized yeast than from feeding Na selenite. Whereas from selenized yeast, about 94 % was incorporated into seleno-amino acids, mainly selonomethionine, and the excess was excreted via feces, excess selenite was excreted via the urinary tract. Both from selenite and from selenized yeast supplementation, serum Se significantly correlated with serum glutathione peroxidase activity, liver Se, kidney Se, loin Se, and pancreas Se [112].

When selenite was administered intravenously (iv) to rats, liver tissue took up Se within minutes, whereas brain tissue did not begin accumulating Se until labeled selonoprotein began appearing in the plasma after 30 min. [113]. Intramuscularly injected selenite to rats got completely redistributed within 2 days, and excretion was 83 % renal and 17 % fecal [114]. In mice, 1–3 h after injection most of the organs attained the highest retention [115]. The uptake of ip-injected Se into various organs of mice within 2 h decreased in the order liver > kidney > spleen, but varied among different Se species. Selenite targeted the liver, whereas Se-methionine yielded highest Se in kidneys and spleen. Se-glutathione, Se-cystine, and Se-cystamine were intermediate [115,116]. After subcutaneous injection, Se-methionine was incorporated into the kidneys, heart, liver, and brain 2–5 times more than selenite. With respect to total burden, the liver took up more than 10 times more of both Se components than the other organs studied. In the brain, selenite preferably moved to myelin, whereas Se-methionine got incorporated in all particulate fractions in about equal levels, at most to the mitochondria [117]. In 2–3-year-old sheep, iv-administered Na-selenate moved to various organs, and appeared rapidly in the bile and saliva. After one day, maximum concentration in blood was reached, and it was excreted first with urine and mainly after 3 days with feces. Accumulation was greatest in the kidneys [118].

Single injections of $BaSeO_4$–emulsions to dairy cows may provide a steady Se source instead of daily feeding, reaching a steady state in blood Se and glutathione peroxidase activity in about 100 days [119].

Excretion proceeds via reduction to selenides from selonocysteine and to selenols from selonomethionine, which are methylated to the major excretory product trimethylselenonium $(CH_3)_3Se^+$ found in urine [120]. After ip injection of trimethylselenonium into rats, renal excretion was significantly quicker in females. Castration of males decreased the whole body retention and the level in kidneys to the levels observed in females of the same age [121]. In experiments with rats, fecal Se excretion was proportional to Se uptake, whereas excess Se was excreted renally in addition. At feeds of more than 1 mg/kg, the renal excretion got overloaded, and excess Se remained in the tissues [100].

When human urine samples were analyzed both by reversed-phase chromatography and ion-pair chromatography, at least 10 Se compounds were separated. From healthy volunteers fed with 1 mg and 2 mg Se bound to yeast, the major urinary metabolite was Se-methyl-*N*-acetylgalactosamine, and minor fraction was Se-methyl-*N*-acetylglucosamine. Larger doses of Se are, however, additionally excreted via methylation as methyl selenol, dimethylselenide, and trimethylselenonium [122].

Though oral uptake is the main pathway for the uptake of Se, uptake from seleniferous coal combustion aerosols have been proved, where Se is concentrated in small, respirable particles as selenite or elemental Se. In 3–4-year-old beagle dogs, inhaled Se was excreted with a half-life of 1.2 days at 70–80 % vs. urine, and just 0.6 % were again exhaled. After 2 h, about 20 % of Se- metal and 2 % of selenious acid was found in the lungs. Major parts had gone to the liver, where it had a half-life of about 40 days [123].

Functions and speciation in living organisms

At the biochemical level, Se has different functions, such as protection of cell membranes from oxidative damage, or interactions with metals and arsenic, and participation in the iodine metabolism. In living cells, high Se doses provoke inhibition of enzymes and blocking of sulfhydryl groups, interfere with

the synthesis of S-containing amino acids, change structures of proteins, and destroy cell membranes. Genotoxic effects on DNA, yeast, and plant mutagenesis have also been reported.

Vertebrates and invertebrates cannot synthesize methionine or selonomethionine, and rely upon fungi, bacteria, algae, and macrophytes [83]. In animals, all essential functions of Se have been associated with selonoproteins, which are redox enzymes that contain selonocysteine in their primary structure. At first, selenophosphate is formed, which reacts with seryl-*t*RNA to selenocysteyl-*t*RNA, in order to insert selonocysteine into a growing polypeptide chain [113]. All three iodothyronine deiodinases are also selonoproteins. Selonoprotein P is an extracellular protein that contains most of the Se in plasma. It is synthesized and secreted by most tissues, but predominantly produced by the liver and transports Se to the brain. Its concentration is sensitive to the Se nutritional status [113].

Four different Se-dependent glutathione peroxidases are known: cellular glutathione peroxidase, gastrointestinal glutathione peroxidase, extracellular or plasma glutathione peroxidase, and phospholipid hydroperoxide glutathione peroxidase [104]. In rats, dietary Se requirements for the full expression of phospholipid hydroperoxide glutathione peroxidase is lower than for cellular glutathione peroxidase. The activities of these peroxidases could be measured by the coupled assay of NADPH oxidation [104]. Selenium as an integral part of glutathione peroxidase is an antioxidant preferentially of the aqueous (cytosolic) compartment, whereas vitamins E and A act as scavengers of free radicals in the lipid compartment of the body [117].

In rats, iv-administered selenite was taken up by red blood cells within several minutes, reduced to selenide by glutathione, and then transported to the plasma, bound selectively to albumin and transferred to the liver. Intact selenate was taken up directly by the liver or excreted into the urine. In the liver, a seleno-sugar (Se-methyl-*N*-acetylselenohexosamine) and its methylated form were identified, which are major urinary metabolites [124]. Single-dose oral administration of selenite or selenate led to formation of dimethylselenide in rat liver at a level 2–13 %, which reached an almost constant level after 2 h. No reaction was noted with the dead tissue homogenate [125].

In human serum, 4 Se-containing proteins with apparent mol masses of 57–74, 46–56, 40–42, and 21–22 kDa could be separated by gel electrophoresis [30]. The incorporation of Se into protein components was different among the chemical forms applied. Selenium from selonomethionine tended to be accumulated at 25–100 kDa, and from selenite and selenate at 10–25 kDa [27]. In subcellular fractions of human liver, 24 kinds of Se-containing proteins were found after gel electrophoresis. They were mostly in the 20–30 and 50–80 kDa range. Major Se-containing protein fractions at 61 and 21 kDa are probably selonoprotein P and glutathione peroxidase. The lowest Se-containing protein of 9.3 kDa only existed in lysosome [29]. In subcellular fractions of human liver, 24 kinds of Se-containing proteins were found after gel electrophoresis. They were mostly in the 20–30 and 50–80 kDa range. Major Se-containing protein fractions at 61 and 21 kDa are probably selonoprotein P and glutathione peroxidase. The lowest Se-containing protein of 9.3 kDa only existed in lysosome [29].

One of the best anticarcinogenic forms of Se was Se-methylselenocysteine, which is a major constituent of plants grown on Se-rich media, but it does not get incorporated into proteins. From this, monomethylated Se was the major excretory product. Benzylselenocyanate Φ-CH_2SeCN, various isomers of xylyl-bis(selenocyanate) NC-Se-CH_2-Φ-CH_2SeCN, and even K-selenocyanate KSeCN are active in chemoprevention of cancer in the initiation phase. Triphenylselenonium chloride Φ_3SeCl, fed orally, gave the best ratio of efficacy to toxicity for any Se compound tested to date. Tissue levels were increased only slightly. As a lipophilic cation, it presumably gets accumulated in mitochondria and has a greater metabolic stability [120].

Though elemental Se is generally inert, except for some bacteria, nanoparticles of elemental red Se have recently been found to have more effect on the activity of glutathione peroxidase than selonomethionine or selenite, isolated from broiler chick kidneys in vitro [126].

Selenium interacts with a number of toxic metals, such as Pb, Ag, Tl, As, Bi, and Cd, and renders these substances less toxic. Therapeutical use of Se compounds has, therefore, been discussed as antidotes against metal toxicities. A direct reaction between the metal and selenide can lead to the for-

mation of insoluble or stable selenides in vivo. In livers of marine mammals (whales, seals) and cormorants living far from man-made sources of pollution, granules correspond to the mineral HgSe tiemannite were identified. Contrary to this, mercury in fish occurs almost completely in organic (methylated) forms. In fish liver, Se occurs in large excess over Hg. Tuna and swordfish have a similar Hg dietary intake like whales or cormorants, but they can additionally excrete via the gills into the water, which is not possible for mammals and seabirds [127].

Aquaculture and fisheries

Zooplankton, benthos, and deposit feeders and even fish ingest most of their Se via nutrition, and not via the water phase. Aquatic biota are more tolerant toward high Se than terrestrials.

For *Daphnia pulex* and *Daphnia magna*, kept under bacteria-free conditions at 20 °C, less than 0.1 μg/l Se is essential, and 1 μg/l is sufficient to satisfy minimal needs (bacteria would mediate uptake from sediments and seston). Culture lines need 0.5 μg/l to be maintained at indefinite lifespan. Se-deprived animals undergo cuticle deterioration similar to the last stages of life (early senescence). They lose their antenna on molting [128].

Hemoglobin and glutathione peroxidase are higher in fish erythrocytes than in all other vertebrates. When carps from a Se-deficient area were fed a diet containing 0.15 mg/kg selenate Se or Se yeast, glutathione and catalase activities increased, whereas glutathione-*S*-transferase activity decreased. Selenium yeast was more efficient than selenate, but the yeast may be also a potential source of other microelements such as Cu and Zn [129]. Cyprinidae are less sensitive to high Se levels than others. In fathead minnows, treatment with fish feed up to 15 mg/kg did not significantly inhibit growth, whereas decline in growth was most obvious at 30 mg/kg [130]. Carps could stand TLm values of 72, 50, and 35 mg/l selenite for 24, 48, and 96 h, respectively, with a biological half-life of Se of 28 days [131]. After 50 days cultured in solutions of 0.5, 1, 2, and 5 mg/l, carp bodies contained just 0.9, 1.6, 1.8, and 2.9 mg/kg, which means the concentration factor from solution is not large. The liver is the first target organ, and the kidneys are the second, whereas Se in heart, bone, and muscles remained low [131]. In adult minnows (cyprinidae, 6–8 cm), soluble selenate applied for 7 days via the water phase preferably went to the gut, liver, and kidneys [132].

OCCURRENCE IN FOOD FOR HUMAN NUTRITION

Contrary to farmed animals, Se is not added to human diet in low-Se areas to meet Se needs, except via mineral fertilizers in Finland [38]. According to the Recommended Daily Allowances in the United States, the average daily intake should be 50–200 μg/d, and according to the German Society of Nutrition, it should be 20–100 μg/d. In Britain, the Se Reference Nutrient Intake has been set to 75 μg/d for adult males and to 60 μg/d for adult females [146]. Usually, human diet is much more variable than the diet of farmed animals. Serum Se, and (better) whole blood Se levels reflect the current Se status of the individual, and median levels obtained within a region indicate a general level of supply. The difference between Se concentrations in human plasma and whole blood is about 30 % [135]. Apart from local feeding habits, blood plasma and serum levels of coastal populations are generally higher than from inside the continents, thus, seafood is a very significant source of Se (Table 3).

In Northern Europe, where soil Se is low, meat and fish are the primary sources of Se. In Sweden, significant differences in human plasma Se between fish-consumers (91 μg/l) and non-fish-consumers (80 μg/l) were established [136]. In contrast, in North America where the Se content of locally grown plant foods is often higher, cereal products are supposed to be the major Se sources [138]. Imported wheat flour, rice, soya products, and cocoa may raise the Se levels in human nutrition available in the European market, particularly in Britain [146]. About 80 % of Se is assumed to be absorbed from mixed diets. High-protein diets as well as ascorbic acid enhance the bioavailability of Cu, Zn, and Se [138].

The average Se intake in Sweden was estimated at 38 μg/d, in Britain at 34 μg/d [146], in the Netherlands 67 μg/d, and in Switzerland at 70 μg/d. The average Se intake in Finland increased from 30 μg/d in 1976 to 113 μg/d in 1986, due to the National Supplementation program [38,139]. In Greece, the daily intake of healthy adults was estimated as 110 μg/d from uncooked food, and 95 μg/d based on uncooked + cooked food [137]. The nutritional status of the Austrian population is regarded to be low, but still adequate. The daily Se intake has been determined to be within the range 27–68 μg/d, with a mean of 48 μg/d [140].

In Brazil, in the districts of higher fish and pork consumption, whole blood Se was also significantly higher [102]. Within an unpublished investigation series of the author, median whole blood Se levels of females in Vienna sampled in 1988/89 were 66 μg/l (range 27–111 μg/l), which was rather low (see Table 3).

The contribution of oil, fats, and sweets to the Se intake is negligible [146].

Beverages

Whereas most potable and mineral waters are very low in Se, much more Se may be present in commercially available fruit- and vegetable juices, like 110–120 μg/l found in Germany in 1988. Belgian beers are reported to contain 0.2–15.2 μg/l [74].

The Se content of wine is usually greater than in potable water from the same location. Data from various parts of grapevines in Germany show that Se is presumably taken only by the roots (that means, no uptake from atmospheric deposition, contrary to moss), and moves to the leaves and partially into the stems. Especially in the grapes, the content is very low. Red wines contain some more Se because of different ways of processing. Further, the soil composition is of some influence. Thus, German red wine from a soil of 0.24 μg/g has an average of 0.79 μg/l, whereas another German red wine grown on calcareous soil with only 0.09 mg/kg Se, has an average of 0.40 μg/l [74]. Contamination of imbottled wine from red ruby glass, which has been colored with up to 0.4 % Se, within 1 year, was about 1–2 μg/l.

In Britain, Se intake from beverages has been estimated as low as 3 % of total [146].

Vegetables and cereals

The Se content of the flora varies significantly with the geological origin of the soil, its pH, plant species, plant age, and protein content. In Central Europe, the flora grown on neutral loess soils contains more Se than on acidic soils, because the bioavailability of soil Se increases significantly with pH. Intense fertilization, particularly with mineral fertilizers, led to a decline of Se levels in the crops because of dilution effects [147]. Vegetables rich in starch and sugar are generally poor in Se. Mustard, caraway, cabbage, broccoli, garlic, and mushrooms supply Se to the food chain [141].

The Se contents of wheat grain, wheat flour, and potato samples from Sweden, Germany, Scotland, and Norway were only 0.009–0.034 mg/kg. Turkish wheat was higher, at 0.072 mg/kg [139]. A recent screening of cereals in Austria revealed Se within the range <0.004 to 0.050 mg/kg, at a mean of 0.024 mg/kg. Barley grown at a chernozem soil near the Hungarian border in 1992 had a mean of 0.046 mg/kg [44]. Potatoes grown in Austria also range between 0.020–0.040 mg/kg dw. Addition of sodium selenate to inorganic fertilizers of the NP or NPK type aiming to enhance Se levels in cereals has been done in Finland [38], and was also tried in field and pot experiments in Austria [71]. Utilization rates from pots were significantly higher than from the fields [71]. Cereals and yeast metabolized the added selenate at more than 60 % to yield selonomethionine-containing proteins, minor amounts have been found as selenite and selenate. This was proved by enzymatic hydrolysis (pronase or protease + lipase), anion chromatography, in for example, citrate buffer, and ICP–MS detection [8,142]. Similarly, in water culture, corn and barley metabolized added selenite preferably to selonomethionine [84].

Contrary to the low levels encountered in Europe, the Se content of wheat grains from Colorado ranged within 0.3–1.2 mg/kg and was not affected by ammonium nitrate fertilization [143]. Maximum values for corn have been reported from seleniferous soils in South Dakota, containing 29 mg/kg Se [70]. Among vegetables, *A. bisulcatus* and broccoli strongly accumulate selenate from soil, whereas tomatoes react moderately. Astragalus had the highest tissue Se concentration and Se volatilization rates, but in terms of Se removal from soil, broccoli was more effective [15]. Some Se is methylated and thus volatilized, proportional to plant tissue concentration and competing with microbial acitivity. Metabolization of Se in seleniferous cabbage lead to Se-methylselenocysteine, Se-methylselenocysteine selenoxide, and selenocystathionine, whereas selenocystine and selonopeptides were negligible. Higher Se content can be also be expected in grass, rape, and linseeds, which are used as basic components for animal nutrition.

Among Se accumulators, garlic grown on seleniferous soil in China contained 205 mg/kg dw, of which 85–95 % were water soluble at 90°, as a low-molecular weight compound. Reversed-phase chromatography and electrospray MS identified the Se species to be γ-glutamyl-Se-methylselenocysteine $HOOC–CH(NH_2)–CH_2–CH_2–CO–NH-CH(COOH)–CH_2–Se–CH_3$ [24]. Se-accumulating mushrooms (*Albatrellus pes-caprae* and *B. edulis*) contained mostly low-molecular-weight Se compounds of about 6 kDa [144]. In extracts from mushroom samples (*B. edulis* from Ohio), Se was primarily associated with a fraction between 2.9–3.2 kDa and a low-molecular-weight fraction. Additionally, about 10 % of Se in the NaOH extraction was found associated with a fraction at 50 kDa 10.5 % [145].

Meat and dairy products

In Europe, addition of Se to feed mixtures for farmed animals generally increases the Se level in kidneys, liver, and eggs [141]; however, feeding practices are not easily traceable to products sold in the market. In Finland, Se supplementation via mineral fertilizers has increased Se in meat at 30–50 %, and in milk at 30 % [38].

Within a pilot study of the author (Table 4), meat and inner organs sold in Austria have been investigated. Pork Se was highest, because feedstuffs for pigs contain higher Se levels than others (see Table 2). Meat from calves and free-living deer, as well as liver from free-living sheep, differed widely. As in all studies, Se in kidney was much higher than in liver samples, although all metabolic studies with animals in the lab (mainly, rats and mice) resulted in liver as the primary target of orally fed Se.

Similar data from Denmark are frequently in the same range as those from Austria. Differences in beef-Se and veal-Se may be explained from different feeding habits. Horses and deer were not included in the Danish diet. Fish muscle contains about double Se of meat [148]. In Britain (samples from 1993/94), Se in pork (138 μg/kg) was significantly higher than in beef (76 μg/kg), which was significantly higher than in lamb (38 μg/kg). Liver ranged from 190–1350 μg/kg, and kidney from 780–2000 μg/kg [146].

In Finland, Se content in cattle muscle and liver samples from organic production was lower than from conventional farming, due to supplementation of feedstuffs, whereas for pigs, the data were overlapping [38]. In Sweden, where Se-supplemented feedstuffs have been used in animal farming since the 1980s, a positive correlation between Se concentration in the liver and increasing age was found in 3 out of 12 counties [103]. Whereas no local differences in beef from Finnish cows were noted (mean 0.46 mg/kg; range 0.17–0.95 mg/kg), local differences in muscles from free-living reindeer were noted (Lapland: 2.21 mg/kg, range 1.34–4.21 mg/kg; Ostrobothnia: 0.34 mg/kg; range 0.17–0.71 mg/kg) [149].

Table 4 Selenium in food from farmed animals in Austria, 2003/2004.

Se µg/kg wet wt.	Muscle		Liver		Kidney	
Veal	**25**	4–64	X		X	
Beef	**18**	8–26	**258**	205–291	**1072**	813–1487
Deer	**45**	17–83	X		X	
Sheep/lamb	X		**429**	184–445	X	
Horses	**62**	49–93	X		X	
Pork	**84**	66–106	**538**	351–689	**1682**	1494–1750

Similar data from Denmark, period 1993–97 [148]

Se µg/kg wet wt.	Muscle		Liver		Kidney	
Veal	**84**	<40–135	**224**	86–546	**1370**	931–1380
Beef	**93**	<40–148	**241**	65–581	**1270**	425–1730
Sheep/lamb	**61**	<40–111				
Pork	**115**	63–210	**508**	65–892	**1930**	1510–2900
Chicken	**124**	71–177	**455**	352–567		
Cod	**297**	193–412				
Flounder	**192**	40–374				
Herring	**294**	236–439				
Trout	**187**	117–285				
Eggs	**242**	171–326				

The liver of wild animals is an excellent reflection of the dietary status. Livers of African buffalos from the Kruger National Park indicated neither excess nor deficiency for Se, Co, and Mn [150]. Liver tissues of free-living moose of 0.5–14.5 years of age have been collected in Sweden to monitor bioavailable Se in natural vegetation. The medians ranged from 90–290 µg/kg wet wt, which is less than for farmed beef.

In cow milk, the lowest Se levels have been reported in New Zealand, but it could be raised by Se supplementation to 8–10 µg/l. For Friesian cows with a daily intake of just 0.5 mg Se per day from pasture, Se transfer from selenized yeast was 2–4 times more effective than from selenate solutions. The milk Se to blood Se ratio was 0.32 for selenized yeast, and just one-half for selenate supplementation; thus, blood Se does not necessarily indicate milk Se. Single subcutaneous injection of $BaSeO_4$ emulsions provided a steady Se source, reaching a steady state after 100 days, instead of feeding Na-selenate or Se yeast daily. Selenium treatment had no significant effect on milk production. 71 % of whole-milk Se was recovered in casein [119,151]. British cheese (Stilton, cottage cheese) of reduced fat content almost doubled in Se levels [146], compared with usual quality.

In baby nutrition, Se and I from milk are readily absorbed [151]. Contrary to cows, higher Se plasma levels in women do not necessarily lead to higher levels in human breast milk, which is concluded from Japanese data [152] (Table 5).

Table 5 Selenium in milk [119,152–154].

Human	16.8 µg/l	Omnivorous, USA
	22.2	Vegetarian, USA
	23	Japan, 1987
Cow (whole milk)	2	New Zealand, 2001
	10.9 ± 0.3	Belgium, 1991
	5.4–11.7	Hungary, 1989
	11–17	Netherlands, 1989
	15 (range 7–34)	Britain, 1993
	11.1–26.0	Spain, 2003
	17.0 ± 5.0	Greece, 1987
	21.5 ± 2.3	Ankara, Turkey, 2000
	22.4	USA, 1990
	25.7 ± 5.3	India, 1993
	28.2 ± 2.9	Izmir, Turkey, 2000
	69.4 ± 7.4	Samsun, Turkey, 2000

Fish and seafood

In aquatic ecosystems, Se availability to higher trophic levels gets regulated primarily by the Se content of food items, and much less from the surrounding water [87]. Accumulation, low excretion, and low sensitivity to Se may explain higher Se levels in seafood than from terrestrial origin. Thus, fish meal as a basic component for feedstuffs contains 0.5–1.4 mg/kg and can easily exceed the permitted threshold value.

As fish muscle may contain about double the Se of meat, Se levels in fish have been found inverse proportional to fat content [38,148]. Unlike for terrestrial vertebrates, the tissue distribution was found as liver > stomach > heart > muscle > kidney [155]. In mussels, the tissue distribution was in the order gill > intestine > adductor > mantle > foot [156].

The main fraction of Se in fish muscle, as well as in selected human and animal tissues and chicken eggs, was water-extractable (57 % for fish on the average). In this extract, trichloro-acetic acid precipitate took about 10 % of the Se. Selenate was more extractable with water than other forms. Contrary to this, molluscs and crustaceans contain Se mainly in a non-water-extractable form, which may be attributable to nonpolar proteins or lipids [157]. Most of the Se in the mussel and fish tissues was found associated with proteins as selonocysteine. In fish, 14–36 % of Se was present as selenate, but there is no reported evidence that this selenate was formed in the tissues from other compounds [156].

Vegetarian and omnivorous diet, diabetes

Whereas vegans deny food of animal origin in general, lactovegetarians take plant foods + dairy products, and lactoovovegetarians take plant foods + dairy products + eggs.

As animal foods and seafoods (fish, liver, kidneys) generally have higher Se concentrations than do plants, omnivores tend to consume diets higher in Se than do vegetarians; the most striking differences have been reported from Sweden [138]. Just in North America and some areas in China, where the Se content of locally grown plant foods is often higher, cereal products are frequently major Se sources. In Europe, animal foods are assumed to supply 69 and 75 % of the Se demand of women and men, respectively [141]. In Finland, Se supplementation via mineral fertilizers has doubled the Se content of wheat and rye, whereas vegetables and eggs remained about constant [38].

Diabetes is unlikely to effect Se levels in blood serum, but as diabetic diet contains more proteins and fewer sweets and fat, it contains significantly more Se than controls in low-level countries. Thus,

the daily Se intake for German diabetic children was determined as 61 μg/d, and just 36 μg/d for controls [164].

SELENIUM IN FOOD PROCESSING AND FOOD STORAGE

Commercial animal feedstuffs usually contain added Na-selenite, and are kept at low humidity (standard humidity is 12 % water) to prevent microbial degradation. Some organic compounds, which are usually met in food and living biota, such as ascorbic acid and oxalate, may gradually reduce major parts of added selenite, presumably to elementary Se [97]. Contrary to low stability of selenite/ascorbic acid mixtures in feeds and extractant solutions in vitro, enhancement of the absorption and utilization of selenite ingested together with ascorbic acid has been reported [138], which should require further investigations.

In milling and processing, grains and cereals loose about 10 % of their Se content due to local heating and volatilization [158].

In the production of green vegetable juices, blanching extracts major parts of total Se, whereas sterilization destroys organic Se compounds without Se losses [159]. Selenium (and also iodine) were weakly retained in vegetables, starch foods, meat, fish, and eggs, whatever cooking method was employed [160]. For example, Se retention after boiling in distilled water was just 8 % for lentils, 26 % for cabbage, 41 % for cauliflower, 52 % for carrots, until they were done. Eggs retained 55 % of their Se after 3 min boiling, and 38 % after 5 min frying [160]. Other authors noted only 10–30 % losses of Se during boiling of noodles and vegetables [137]. For seafood, however, most common cooking techniques (baking, broiling) did not result in major Se losses [161].

Se losses during grilling of pork obtained at various temperatures were similar, because higher temperatures led to shorter grilling times to be ready [162]. In the baking process, some Se may be lost, because the Maillard reaction of selonomethionine and glucose yields volatile seleniferous compounds [163]. Smoking and cooking may convert selenide and selenite to selenate [161].

Due to losses from cooking, in Greece the daily intake of healthy adults was estimated as 110 μg/d from uncooked food, and 95 μg/d based on uncooked + cooked food [137].

In extracts of feeds in the physiological range, Se speciation was cleary unstable. Anion chromatography of an acetate extract of a protein concentrate for piglets as well as of a mineral premix for pigs yielded 2–3 peaks, whereas in the water and phosphate buffer extract just 1 Se 2 peaks appeared. The first peak could have resulted from a seleno-organic compound as a result of a metabolization reaction during storage of the extract overnight, whereas the subsequent peaks clearly were selenite, respectively selenite + selenate [97].

REFERENCES

1. K. Forchhammer and A. Boeck. *Naturwissenschaften* **78**, 497–504 (1991).
2. M. Sager. In *Environmental Contamination*, J. P. Vernet (Ed.), pp. 403–476, Elsevier, Amsterdam (1993).
3. K. S. Dhillon and S. K. Dhillon. *Adv. Agron.* **79**, 119–184 (2003).
4. G. Banuelos, N. Terry, D. L. LeDuc, E. A. H. Pilon-Smits, B. Mackey. *Environ. Sci. Technol.* **39** (6), 1771–1777 (2005).
5. M. Sager. In *Analytiker Taschenbuch Band 12*, pp. 257–312, Springer, New York (1994).
6. C. E. Sieniawska, R. Mensikow, H. T. Delves. *J. Anal. At. Spectrom.* **14**, 109–112 (1999).
7. A. M. Featherstone, A. T. Townsend, G. A. Jacobson, G. M. Peterson. *Anal. Chim. Acta* **512**, 319–327 (2004).
8. M. Stadlober, M. Sager, K. J. Irgolic. *Food Chem.* **73**, 357–366 (2001).
9. K.-S. Park, S.-T. Kim, Y. M. Kim, Y.-J. Kim, W. Lee. *Bull. Korean Chem. Soc*. **24** (3), 285–290 (2003).

10. C. A. Shand and A. M. Ure. *J. Anal. At. Spectrom.* **2**, 143 (1987).
11. B. Sampson. *J. Anal. At. Spectrom.* **2**, 447 (1987).
12. B. Welz, G. Schlemmer, J. R. Mudakavi. *J. Anal. At. Spectrom.* **3**, 695 (1988).
13. M. A. Deschuytere, H. J. Robberecht, H. Deelstra. *Bull. Soc. Chim. Belg.* **97**, 99 (1988).
14. V. Krivan and M. Kückenwaitz. *Fresnius' J. Anal. Chem.* **342**, 692 (1992).
15. E. C. Duckart, L. J. Waldron, H. E. Doner. *Soil Sci.* **53**, 94 (1992).
16. J. Piwonka, G. Kaiser, G. Tölg. *Fresnius' J. Anal. Chem.* **321**, 225 (1985).
17. A. Wolf, P. Schramel, G. Lill, H. Hohn. *Fresnius' J. Anal. Chem.* **317**, 512 (1984).
18. M. Bedrossian. *Anal. Chem.* **56**, 311 (1984).
19. G. K. H. Tam and G. Lacroix. *J. AOAC Int.* **65**, 647 (1982).
20. M. Dermelj, A. R. Byrne, M. Franko, B. Smodis, P. Stegnar. *J. Radioanal. Nucl. Chem. Lett.* **10**, 91 (1986).
21. J. L. Gomez-Ariza, J. A. Pozas, I. Giraldez, E. Morales. *J. Chromatogr., A* **823**, 259–277 (1998).
22. V. Diaz Huerta, L. Hinojosa Reyes, J. M. Marchante-Gayon, M. L. Fernandez Sanchez, A. Sanz-Medel. *J. Atom. Abs. Spectrom.* **18**, 1243–1247 (2003).
23. J. Zheng, M. Ohata, N. Furuta, W. Kosmus. *J. Chromatogr., A* **874** (1), 55–64 (2000).
24. S. McSheehy, W. Yang, F. Pannier, J. Szpunar, R. Lobinski, J. Auger, M. Potin Gautier. *Anal. Chim. Acta* **421**, 147–153 (2000).
25. I. Ipolyi, W. Corns, P. Stockwell, P. Fodor. *J. Autom. Methods Manage. Chem.* **23** (6), 167–172 (2001).
26. J. Alexander and J. Aaseth. *Toxicology* **21**, 179–186 (1981).
27. Z.-J. Wang, J. Zhou, A. Peng. *Biol. Trace Elem. Res.* **33**, 135–143 (1992).
28. C. N. Ferrarello, M. R. Fernandez de la Campa, J. F. Carrasco, A. Sanz-Medel. *Spectrochim. Acta, Part B* **57**, 439–449 (2002).
29. C. Y. Chen, J. J. Zhao, P. Q. Zhang, Z. F. Chai. *Anal. Bioanal. Chem.* **372** (3), 426–430 (2002).
30. Y. Gao and Z. Wang. *Fresnius' J. Anal. Chem.* **367** (1), 60–64 (2000).
31. G. A. Cutter. *Science* **217**, 829 (1982).
32. S. G. Jiang, D. Chakraborti, F. Adams. *Anal. Chim. Acta* **196**, 271 (1987).
33. M. Dall'Aglio, E. Ghiara, W. Proietti. *Rend. Soc. Ital. Min. Petrol.* **34**, 591 (1978).
34. S. R. Krajnov, Z. G. Gudz, V. P. Zakutin, N. I. Enikeev, S. M. Pribytkova. *Geokhim.* **VOL.?** 359 (1983).
35. D. Wang, G. Alfthan, A. Aro, P. Lahermo, P. Väänänen. In *Trace Elements in Health and Disease*, pp. 49–56, Royal Society of Chemistry, Cambridge (1991).
36. M. K. Saiki, M. R. Jennings, W. G. Brumbaugh. *Arch. Environ. Contam. Toxicol.* **24**, 307–319 (1993).
37. T. W. May, M. J. Walther **et al.** *Environ. Monit. Assess.* **72**, 176–206 (2001).
38. M. Eurola, G. Alfthan, A. Aro, P. Ekholm, V. Hietaniemi, H. Rainio, R. Rankanen, E.-R. Venäläinen. *Results of the Finish Selenium Monitoring Program 2000–2001*, MTT Agrifood Research Finland, Jokioinen (2003).
39. T. Bronikowski, W. Pasiuk-Bronikowska, M. Ulejczyk, R. Nowakowski. *J. Atmos. Chem.* **35**, 19–31 (2000).
40. K. N. Allen. *J. Environ. Qual.* **20**, 865 (1991).
41. O. H. Danneberg. *Mitt. Österr. Bodenkundl. Ges.* **57**, 7–24 (1999).
42. X. Wu and J. Låg. *Acta Agric. Scand.* **38**, 271–276 (1988).
43. W. Van Derveer and S. P. Canton. *Environ. Toxicol. Chem.* **16** (6), 1260–1268 (1997).
44. M. Sager. *Bericht über Schadstoffbelastungen der Parndorfer Platte vor dem Bau der Ostautobahn*, Bericht der Landwirtschaftlich Chemischen Bundesanstalt, Wien (1992).
45. M. Sager. *Toxicol. Environ. Chem.* **71**, 159–183 (1999).
46. J. W. Kaakinen, R. M. Jorden, M. H. Lawasani, R. E. West. *Environ. Sci. Technol.* **9**, 862–872 (1975).

47. J. J. Rowe and E. Steinnes. *Talanta* **24**, 433 (1977).
48. H. A. Van Der Sloot, D. Hoede, T. J. L. Klinkers, H. A. Das. *J. Radioanal. Chem.* **71**, 463 (1982).
49. D. Kut and Y. Sarikaya. *J. Radioanal. Chem.* **62**, 161 (1981).
50. K. Augustin-Gyurits and E. Schroll. Report of the Federal Research Centre Arsenal, Vienna, Austria (1989).
51. A. Wadge and M. Hutton. *Proc. Int. Conf. Heavy Met. Environ.* Athens pp. 562–563 (1985).
52. J. L. Fio and R. Fuji. *Soil Sci. Soc. Am. J.* **54**, 363–369 (1990).
53. F. Seby, M. Potin Gautier, G. Lespes, M. Astruc. *Sci. Total Environ.* **207**, 81–90 (1997).
54. T. T. Chao and R. F. Sanzolone. *Soil Sci. Soc. Am. J.* **53**, 385–392 (1989).
55. D. A. Martens and D. I. Suarez. *Environ. Sci. Technol.* **31**, 133–139 (1997).
56. I. Heninger, M. Potin Gautier, M. Astruc, L. Galvez, V. Vignier. *Chem. Spec. Bioavail.* **10**, 1–9 (1998).
57. M. Bujdoš, J. Kubova, V. Streško. *Anal. Chim. Acta* **408**, 103–109 (2000).
58. Y. Q. Zhang and J. N. Moore. *Appl. Geochem.* **12**, 685–691 (1997).
59. M. Sager. *Bodenkultur* **53** (2), 83–103 (2002).
60. Y. Qiang Zhang and W. T. Frankenberger. *Intern. J. Environ. Anal. Chem.* **83** (4), 315–326 (2003).
61. M. T. Wright, D. R. Parker, C. Amrhein. *Environ. Sci. Technol.* **37**, 4709–4716 (2003).
62. M. M. Abrams and R. G. Burau. *Commun. Soil Sci. Plant Anal.* **20**, 221–237 (1989).
63. M. M. Abrams, R. G. Burau, R. J. Zasoski. *Soil Sci. Soc. Am. J.* **54**, 979–982 (1990).
64. G. S. Klebanov and N. A. Ostankevich. *Zh. Prikl. Khim.* **33**, 1957–1961 (2000).
65. M. L. Berrow and A. M. Ure. In *Occurrence and Distribution of Selenium*, M. Ihnat (Ed.), pp. 213–242, CRC Press, Boca Raton, FL (1989).
66. M. H. Alemi, D. A. Goldhamer, D. R. Nielsen. *J. Environ. Qual.* **20**, 89–95 (1991).
67. N. J. Barrow and B. R. Whelan. *J. Soil Sci.* **40**, 29–37 (1989).
68. M. P. Arvy. *Commun. Soil Sci. Plant Anal.* **23**, 1397 (1992).
69. S. M. Nye and P. J. Peterson. In *Trace Substances in Environmental Health*, Vol. 9, D. D. Hemphill (Ed.), pp. 113–121, University of Missouri, Columbia (1975).
70. M. A. Arthur, G. Rubin, P. G. Woodbury, R. E. Schneider, L. H. Weinstein. *Environ. Toxicol. Chem.* **11**, 1289–1299 (1992).
71. M. Sager and J. Hoesch. In *International Symposium on the Fusion Technology of Geosystem Engineering, Rock Engineering and Geophysical Exploration*, T.-S. Lee, S.-Y. Choi, D. S. Lee, H. K. Moon, S. Jeon (Eds.), pp. 479–492, CIR Communications, Seoul (2003).
72. T. Yläranta. *Ann. Agric. Fenn.* **22**, 139–151 (1983).
73. T. Yläranta. *Ann. Agric. Fenn.* **22**, 164–174 (1983).
74. H. R. Eschnauer, F. Alt, J. Messerschmidt, G. Tölg. *Fresnius' J. Anal. Chem.* **332**, 874 (1989).
75. K. E. Panter, L. F. James, H. F. Mayland. *Vet. Hum. Toxicol.* **37** (1), 30–32 (1995).
76. M. Montes-Bayon, D. L. LeDuc, N. Terry, J. A. Caruso. *J. Anal. At. Spectrom.* **17** (8), 872–879 (2002).
77. Wu Lin and Guo Xun. *Ecotoxicol. Environ. Saf.* **51**, 22–27 (2002).
78. C. Eriksson and C. Pedrós-Alió. *Can. J. Microbiol.* **36**, 475–483 (1990).
79. P. Kiffney and A. Knight. *Arch. Environ. Contam. Toxicol.* **19**, 488–494 (1990).
80. A. Moede, R. W. Greene, D. F. Spencer. *Environ. Exper. Bot.* **20**, 207–212 (1980).
81. G. F. Riedel and J. G. Sanders. *Environ. Toxicol. Chem*. **15** (9), 1577–1583 (1996).
82. M. Williams, R. S. Ogle, A. W. Knight, R. G. Burau. *Arch. Environ. Contam. Toxicol.* **27**, 449–453 (1994).
83. J. Alaimo, R. S. Ogle, A. W. Knight. *Arch. Environ. Contam. Toxicol.* **27**, 441–448 (1994).
84. C. J. Cappon. *Sci. Total Environ.* **100**, 177–205 (1991).
85. Y. Zhang, Z. A. Zahir, W. T. Frankenberger. *J. Environ. Qual*. **33**, 559–564 (2004).
86. L. Guo, W. T. Frankenberger Jr., W. A. Jury. *Water Res.* **35**, 663–669 (1999).

87. G. L. Bowie, J. G. Sanders, G. F. Riedel, C. C. Gilmour, D. L. Breitburg, G. A. Cutter, D. B. Porcella. *Water, Air, Soil Pollut.* **90**, 93–104 (1996).
88. G. F. Riedel, J. G. Sanders, C. C. Gilmour. *Aquat. Microb. Ecol.* **11**, 43–51 (1996).
89. T. Tuve and H. H. Williams. *J. Biol. Chem.* **236** (2), 597–601 (1961).
90. R. S. Dungan and W. T. Frankenberger. *J. Environ. Qual.* **27**, 1301–1306 (1998).
91. R.S. Oremland, **et al.** *Appl. Envir. Microbiol.* **55**, 2333–2343 (1989).
92. J. W. Doran and M. Alexander. *Soil Sci. Soc. Am. J.* **41**, 70 (1977).
93. T. Yläranta. *Ann. Agric. Fenn.* **21**, 103 (1982).
94. P. H. Masscheleyn, R. D. Delaune, W. H. Patrick. *J. Environ. Qual.* **20**, 522 (1991).
95. H. Yamada, T. Miyamura, A. Yasuda, T. Hattori, K. Yonebayashi. *Soil Sci. Plant Nutr.* **40**, 49–56 (1994).
96. R. Zieve and P. J. Peterson. *Sci. Total Environ.* **19**, 277 (1981).
97. M. Stadlober, M. Sager, K. J. Irgolic. *Bodenkultur* **52** (4), 313–322 (2001).
98. R. Forrer, K. Gautschi, H. Lutz. *J. Trace Elem. Health Dis.* **5**, 101–115 (1991).
99. P. R. Paullicks, E. Vertesy, M. Kirchgessner. *Proc. Soc. Nutr. Physiol.* **6**, 142 (1997).
100. W. Windisch, S. Gabler, M. Kirchgessner. *Proc. Soc. Nutr. Physiol.* **6**, 143 (1997).
101. R. Van Cauwenbergh, H. Robberecht, H. Deelstra. *J. Trace Elem. Electrolytes Health Dis.* **4,** 215 (1990).
102. S. Da Cunha, F. Albanesi, F. Manes, D. S. Antelo, M. Mirando de Souza. *Sci. Total Environ.* **301** (1–3), 51–54 (2003).
103. V. Galgan and A. Frank. *Sci. Total Environ.* **172**, 37–45 (1995).
104. L. X. Gen, H. M. Dann, D. A. Ross, C. Wen-Hsing. *J. Nutr.* **128**, 130–135 (1998).
105. M. Mihailovič, G. Matič, P. Lindberg, B. Žigič. *Biol. Trace Elem. Res.* **33**, 63–69 (1992).
106. J. L. Martin and J. A. Hurlbut. *Phosphorus Sulfur* **1**, 295–300 (1976).
107. G. Dini, F. Martini, I. Stendardi, F. Bernardini, F. Franconi. *Exp. Mol. Pathol.* **36**, 326–332 (1982).
108. G. H. Heinz, D. J. Hoffman, L. G. Gold. *Arch. Environ. Contam. Toxicol*. **17**, 561–568 (1988).
109. P. D. Whanger, N. D. Pedersen, J. Hatfield, P. H. Weswig. *Proc. Soc. Exp. Biol. Med.* **153**, 295–297 (1976).
110. S. Gabler, W. Windisch, M. Kirchgessner. *Proc. Soc. Nutr. Physiol.* **6**, 138 (1997B).
111. S. O. Knowles, N. D. Grace, K. Wurms, J. Lee. *J. Dairy Sci.* **82**, 429–437 (1998).
112. D. C. Mahan and N. A. Parrett. *J. Anim. Sci*. **74**, 2967–2974 (1996).
113. R. F. Burk, K. E. Hill, A. K. Motley. *J. Nutr.* **133**, 1517S–1520S (2003).
114. S. Gabler, W. Windisch, M. Kirchgessner. *Proc. Soc. Nutr. Physiol.* **6**, 137 (1997 A).
115. M. Czauderna and M. Rochalska. *J. Radioanal. Nucl. Chem.* **129** (2), 425–433 (1989 A).
116. M. Czauderna and M. Rochalska. *Appl. Radiat. Isot.* **40** (2), 177–181 (1989 B).
117. J. Clausen. *Biol. Trace Elem. Res.* **28** (1), 39–45 (1991).
118. J. Dejneka, R. Nowosad, J. Simoni. *Polskie Arch. Wet.* **21** (2), 237–248 (1979).
119. N. D. Grace, K. Ankenbauer-Perkins, A. M. Alexander, R. M. Marchant. *N. Z. Vet. J.* **49** (1), 24–28 (2001).
120. H. E. Ganther and J. R. Lawrence. *Tetrahedron* **53** (36), 12299–12310 (1997).
121. J. Pařizek, J. Kalouskova, L. Pavlik, M. Styblo. *Biol. Trace Elem. Res.* **34**, 257–263 (1992).
122. B. Gammelgaard and L. Bendahl. *J. Anal. At. Spectrom.* **19**, 135–142 (2004).
123. S. H. Weissman, R. G. Cuddihy, M. A. Medinsky. *Toxicol. Appl. Pharmacol.* **67**, 331–337 (1983).
124. K. T. Suzuki and Y. Ogra. *Food Add. Contam.* **19** (10), 974–983 (2002).
125. K. Nakamuro, Y. Sayato, Y. Ose. *Toxicol. Appl. Pharmacol.* **39**, 521–529 (1977).
126. B.-H. Xu, Z.-R. Xu, M.-S. Xia, C.-H. Hu, Y.-S. Deng, L. Xiong. *Wuhan Univ. J. Nat. Sci.* **8** (4), 1167–1172 (2003).
127. M. Nigro and C. Leonzio. *Mar. Ecol.: Prog. Ser.* **135**, 137–143 (1996).
128. K. I. Keating, B. Caballero Dagbusan. *Proc. Nat. Acad. Sci. USA* **81**, 3444–3437 (1984).

129. A. Jovanovič, G. Grubor-Lajsič, N. Djukič, G. Gardinovacki, A. Matič, M. Spasič. *Crit. Rev. Food Sci. Nutr.* **37** (5), 443–448 (1997).
130. R. S. Ogle and A. W. Knight. *Arch. Environ. Contam. Toxicol.* **18**, 795–803 (1989).
131. T. Sato, Y. Ose, Ts. Sakai. *Environ. Pollut.* **21**, 217–224 (1980).
132. M. L. A. Cuvin Aralar and R. W. Furness. *Bull. Environ. Contam. Toxicol.* **45**, 775–782 (1990).
133. J. Kvicala, V. Zamrazil, V. Jiranek. *Biol. Trace Element Res.* **71–72**, 31–39 (1999).
134. D. R. Mayer, W. Kosmus, W. Beyer, H. Pogglitsch, K. J. Irgolic. In *Trace and Toxic Elements in Nutrition and Health*, M. Abdullah (Ed.), pp. 41–52, Whiley Eastern, New Delhi (1995).
135. A. Donadini, C. Fiora, R. Regazzini, D. Perini, C. Minoia. *Clin. Exper. Dermat.* **17**, 214–216 (1992).
136. B. G. Svensson, A. Schütz, A. Nilsson, B. Åkesson, S. Skerfving. *Sci. Total Environ*. **126**, 61–74 (1992).
137. M. S. Bratakos, T. F. Zafiropoulos, P. A. Siskos, P. V. Ioannou. *Int. J. Food Sci. Technol.* **23**, 585–590 (1988).
138. R. Gibson. *Am. J. Clin. Nutr.* **59** suppl., 1223–1232 (1994).
139. J. T. Kumpulainen. *J. Trace Elem. Electrolytes Health Dis.* **7**, 107–108 (1993).
140. M. Wilplinger and W. Pfannhauser. *Essentielle Spurenelemente Cr, Cu, Mo, Ni, Se und Zn.* Forschungsprojekt der TU Graz (1997).
141. R. Müller, M. Anke, K. Frobus. *Fresnius Environ. Bull.* **12** (6), 584–588 (2003).
142. V. Diaz Huerta, M. L. Fernandez Sanchez, A. Sanz-Medel. *J. Atom. Abs. Spectrom.* **19**, 644–648 (2004).
143. P. N. Soltanpour, S. R. Olsen, R. J. Goos. *Soil Sci. Am. J.* **46**, 430 (1982).
144. Z. Slejkovec, J. Van Elteren, U. Woroniecka, K. J. Kronn, I. Falnoga, A. R. Byrne. *Biol. Trace Elem. Res*. **75** (1–3), 139–155 (2000).
145. R. G. Wuilloud, S. S. Kannamkumarath, J. A. Caruso. *J. Agric. Food Chem.* **52**, 1315–1322 (2004).
146. M. N. I. Barclay, A. MacPherson, J. Dixon. *J. Food Comp*. *Anal.* **8**, 307–318 (1995).
147. A. Edelbauer and G. Eder. *Bodenkultur* **52** (3), 209–214 (2001).
148. E. H. Larsen, N. L. Andersen, A. Møller, A. Petersen, G. K. Mortensen, J. Petersen. *Food Add. Contam.* **19** (1), 33–46 (2002).
149. P. Kurkela and E. Kaantee. *Zentralbl. Vet. Med. B* **26**, 169 (1979).
150. E. C. Webb, J. B. J. Van Ryssen, M. E. A. Erasmus, C. M. E. McCrindle. *J. Environ. Monit.* **3** (6), 583–585 (2001).
151. S. O. Knowles, J. Lee, N. D. Grace. *Proc. Nutr. Soc. N. Z.* **22**, 174–183 (1997).
152. Y. Hojo. *Sci. Total Environ.* **65**, 85 (1987).
153. R. Inam and G. Somer. *Food Chem.* **69**, 345–350 (2000).
154. P. Cava-Montesinos, M. L. Cervera, A. Pastor, M. De la Guardia. *Anal. Chim. Acta* **481**, 291–300 (2003).
155. W. Maher, M. Deaker, D. Jolley, F. Krikowa. B. Roberts. *Appl. Organomet. Chem.* **11** (4), 313–326 (1997).
156. C. J. Cappon and J. C. Smith. *Arch. Environ. Contam. Toxicol.* **10**, 305 (1981).
157. C. J. Cappon and J. C. Smith. *J. Anal. Toxicol.* **6**, 10 (1982).
158. B. Hakansson, M. Jägerstad, R. Öste, B. Åkesson, L. Jonsson. *J. Cereal Sci.* **6** (3), 269–282 (1987).
159. Z. Min, L. Chun Li, C. Ping. *J. Food Eng.* **62** (4), 393–398 (2004).
160. M. Lamand, J. C. Tressol, S. Villart. *Cah. Nutr. Dietetique* **32** (1), 28–30 (1997).
161. L. Xi, L. Yi, D. F. Jiao, Q. S. Sheng. *Wuhan Univ. J. Nat. Sci.* **8** (1A), 95–98 (2003).
162. J. A. Driskell, D. W. Giraud, J. Sun, S. Joo, F. L. Hamouz, S. L. Davis. *J. Food Qual.* **21** (3), 201–210 (1998).

163. G. J. Wei and C. T. Ho. In *Heteroatomic Aroma Compounds*, ACS Symposium Series 826, G. A. Reineccius and T. A. Reineccius (Eds.), pp. 289–301, American Chemical Society, Washington, DC (2002).
164. K. Bach, H. J. Haas, A. Mathieu. *Pädiatrie Psychologie* **26**, 83–85 (1991).