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Element distribution in *Lactarius rufus* in comparison to the underlying substrate along a transect in southern Norway

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ABSTRACT

Lactarius rufus, the rufous milkcap, is one of the most widespread fungi in northern Europe. 40 samples of *Lactarius rufus* were collected along a 100 km transect in an almost pristine area of southern Norway. Along the transect two mineral deposits occur (a) the Nordli porphyry molybdenum deposit and (b) the Snertingdal Pb occurrence. 53 chemical elements were analysed in *Lactarius rufus* and its substrate, the soil O and C horizon. Of these, 32 elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, Ti, Tl, V and Zn) returned values above the respective detection limits for the majority of samples.

Compared to the soil C-horizon *Lactarius rufus* is strongly enriched (factor > 2 to 72) in K, Rb, Ag, P, S, Na, Cd, Hg, Cu, Zn and Cs. Compared to the soil O horizon Rb, K, P, Cs, Cu, S and Na are the most enriched (factor > 2 to 50) elements in *Lactarius rufus*. Compared to the median of in total 15 plant materials collected along the same transect *Lactarius rufus* is most strongly enriched in Ag (195 times), more than 10 times in Cs, Rb, Na, Cd and 10 to 2 times in K, Hg, P, Cu, Ti, As, S and Zn. Compared to other plants the uptake of Ba, Ca, Mn, Sr, Al and Mo by *Lactarius rufus* is very low. Element correlations between *Lactarius rufus* and the substrate are generally poor. Within *Lactarius rufus* only the major nutrients (K, P, S, Mg) correlate well. Part of the study was to investigate *Lactarius rufus* suitability for biogeochemical exploration; although this mushroom species enriches many elements, it does not provide a good indication of the presence of the mineral occurrences.

1. Introduction

More than 3000 species of fungi are reported to occur in Western Europe (Moser, 1983). Fungi influence biogeochemical processes involving soil, rock and minerals and plant root–soil interface, are involved in the cycling of chemical elements within the soil (Aloupi et al., 2012 and references therein) and have a unique ability to degrade cellulose and lignin (Ruthes et al., 2013).

A remarkable adaptation is the formation of mutualistic partnerships (mycorrhizas) between fungi and land plants. The mycorrhizal fungi lead to changes in the physico-chemical characteristics of the environment around roots, which lead to enhanced weathering of soil minerals and increased metal cation release (Gadd, 2007). *Lactarius rufus*, commonly known under the name “rufous milkcap” belongs to ectomycorrhizal fungi, which form a partnership with Scots pine, *Pinus sylvestris* (Aspray et al., 2006; Krupa and Kozdroj, 2007; Poole et al., 2001; Rudawska and Leski, 2005). Ectomycorrhizal fungi are located outside the root cells of the host plant (Aloupi et al., 2012), forming fungal-roots that consist of a thick fungal mantle. Ectomycorrhizal fungi developing in the tree roots can bind excessive amounts of heavy metals with negatively charged sites on cell walls. In this way mycorrhizae can reduce metal concentrations in plant shoot tissues (Leyval et al., 1997). For temperate and boreal ecosystems, more than 90% of forest tree fine roots are colonized by ectomycorrhizal fungi (Markkola, 1996). Ectomycorrhizae are colonized by different species of bacteria that promote and stimulate mycorrhiza formation through different mechanisms (Krupa and Kozdrój, 2007 and references within; Poole et al., 2001). The ectomycorrhizal mycelium represents the major pathway by which carbon enters the soil (Read and Perez-Moreno, 2003).

A large variation in the consumption of mushrooms exists in Europe; from 5.6 kg in median per capita consumed in the Czech republic (Šišák, 2007) to 0.12 kg in the UK (De Román et al., 2006). A considerable consumption of wild mushrooms in some countries have triggered several studies to determine whether wild mushroom consumption gives rise to health risks (Aloupi et al., 2012; Dogan et al., 2006; Falandysz et al., 2004; Liu et al., 2015; Ouzouni et al., 2007), due to their propensity to accumulate elevated amounts of certain metals and metalloids (e.g., Ag, As, Au, Hg, Se, Sb) (Dunn and Hale, 2007).

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These elements are enriched in addition to the nutrients (e.g. Ca, Cu, Fe, Mg, K, Na and Zn) that are essential for fungal growth and metabolism (Aloupi et al., 2012). However, there seems to be a difference in metal uptake, at least for Au (Borovička et al., 2005, 2010) and Ag (Borovička, 2004), that depends on the type of mushroom analysed, with saprobic fungi taking up metals more readily than ectomycorrhizal fungi.

Two main factors affect metal contents in the fruiting bodies of mushrooms: species-dependence and the chemical composition of the substrate on which the mushroom grows (Aloupi et al., 2012; Cocchi et al., 2006; Kalač et al., 1991; Kalač, 2010; Řanda and Kučera, 2004; Stivje et al., 2004). Fungi play a vital role in the concentration and redistribution of metals in higher plants and soil (e.g. Dunn and Hale, 2007) and yet the fungi/soil relationship is presently not well documented.

Throughout Europe and North America, *Lactarius rufus* is not usually considered an edible species, but it is eaten, mostly in pickled form, for example in Finland (Ohenoja and Koistinen, 1984). It is typical of temperate and boreal forest soils (pine, birch and spruce forests) due to its symbiotic partnership with certain plant species. It has a characteristic dark brick-red colour and a small central raised bump (umbo), that later flattens, eventually acquiring a shallow central depression. This and the colour are easy to recognize, even by non-mycologists. The common occurrence and the ease of recognition makes *Lactarius rufus* a suitable species to collect along a biogeochemical transect. The samples were collected at an average density of 1 site per 81 km^2.

The main lithologies of the study area have been characterized through 61 bedrock samples that have been collected for NGU's litho geochemistry project LITO (for details see: http://www.ngu.no/lito/). The study area is generally divided into four main lithologies that occur along the transect (Fig. 1): Neoproterozic-Silurian sedimentary rocks (SED) which also host the Snertingdal lead mineralisation, Proterozoic mica gneisses (MI GN), Proterozoic granitic gneisses (GR GN) and Permian intrusive rocks (OS RIF) - the Oslo Rift. These intrusive rocks, which include granites and syenites (Lutro, 2001) in the southern part of the transect, host the Nordli Mo deposit (Fig. 1).

In September 2014, the Geological Survey of Norway (NGU) carried out a training course for biogeochemical sampling. The area (Fig. 1) was selected to cover two mineralisations I) the Nordli porphyry molybdenum deposit (Pedersen, 1986), with over 400 million tons of reserves in a hitherto untouched Mo occurrence and II) the Snertingdal lead mineralisation, a c. 2 million tons untouched sandstone-hosted mineralisation (Bjørlykke et al., 1973; Bølviken, 1976). In addition, a lead-poisoned field (Låg et al., 1970) (location 28 in Fig. 1) occurs within the c. 100 km long transect area. The transect is an extension of an earlier transect, leading through Oslo (Reimann et al., 2007) towards the north. Along the Gjøvik transect, soil C and O horizon samples were collected in addition to a large variety of plant materials (fern, horsetail, pine bark, birch, blueberry and cowberry twigs and leaves as well as spruce twigs and needles). Since 2014 was a year in which mushroom growth was good, it also seemed feasible to add on the most common fungus species occurring in the area, *Lactarius rufus*.

One aim of this study is to investigate the suitability of mushrooms for biogeochemical exploration. That is rarely done due to difficulties in distinguishing between the different fungi species, their short growing season, the selective growth locations as well as a requirement of careful handling in the field. Furthermore, the fungus/soil relationship will be studied and element concentrations in *Lactarius rufus* will be compared to those in the soil O and C horizons and the median of all plant materials collected along the transect. This will assist in outlining the specific uptake/rejection mechanism of a common mushroom in comparison to the main plant species occurring along the transect.

2. Methods

2.1. Sampling

The samples were taken in September 2014. In order to conduct a targeted study on the mineralisations, additional samples were taken at these sites. *Lactarius rufus* was collected using vinyl gloves over an area of approximately 500 m^2, in the same area as all other media were collected. *Lactarius rufus* was collected in white Hubco Cloth Soil Sample Bags obtained from Forestry Suppliers, Inc, US. These bags allow the samples to dry without starting to mould.

This study makes several comparisons between *Lactarius rufus* and the soil O— and C-horizons and full sampling descriptions for these two media can be found in Flem et al. (2018). In short, the O-horizon samples were collected as composite samples of 5 sub-samples taken with a steel spade. Live plant material was removed from the top of the sample and any non-organic material was removed from the bottom so that only the uppermost 2–5 cm of the humus and litter layer was retained. The sub-samples were stored in the same cloth sample bags as the mushroom samples. The soil C-horizon samples were collected at one of the O-horizon sub-sample sites. Podzol was the dominant soil type. The median depth to the top of the C-horizon was 30 cm, ranging from 10 to 80 cm. Approximately 2–3 kg of till was collected in contamination-free Rilsan® bags. The sampling tools were thoroughly cleaned (washed in a nearby stream or lake) before moving on to the next location.

2.2. Sample preparation

Samples were air-dried in their respective sample bags. The complete fruiting body of the fungi was used without dividing the specimens into cap and stipe. The dried fruiting body was milled in a Retsch SM 100 cutting mill, which was thoroughly cleaned after each sample. Sample duplicates was inserted as to be invisible for the laboratory between the project samples. All plant materials and the O-horizon samples were prepared in the same way, while the C-horizon samples were sieved to < 2 mm using nylon screening.

2.3. Analysis

All samples were analysed at Bureau Veritas Minerals laboratories in Vancouver, Canada. The fungi, all plant materials and the O-horizon samples were analysed using the standard package 'VG105 Dry Plant Material Analysis' for 5 g splits. The samples were first leached with concentrated nitric acid for 1 h and afterwards digested in a hot water bath for an additional hour. After cooling, a modified aqua regia solution of equal parts of concentrated ACS grade HCl and HNO_3 and demineralised H_2O was added to each sample (6 mL/g) to leach in a hot (95 °C) water bath for 2 h. After cooling, the solution was made up to a final volume with 5% HCl and then filtered. The sample weight to solution volume ratio is 1 g per 20 mL. All solutions were analysed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Elan 6000) for 53 elements (Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Ge, Hf, Hg, In, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Pd, Pt, Rb, Re, S, Sb, Sc, Se, Sn, Sr, Ta, Te, Th, Ti, Tl, U, V, W, Y, Zn, and Zr).

The soil C-horizon samples underwent a modified aqua regia digestion and were analysed for the same 53 elements as above following an aqua regia extraction on 15 g of sample material, for a detailed description of the analytical procedure refer to Flem et al. (2018).

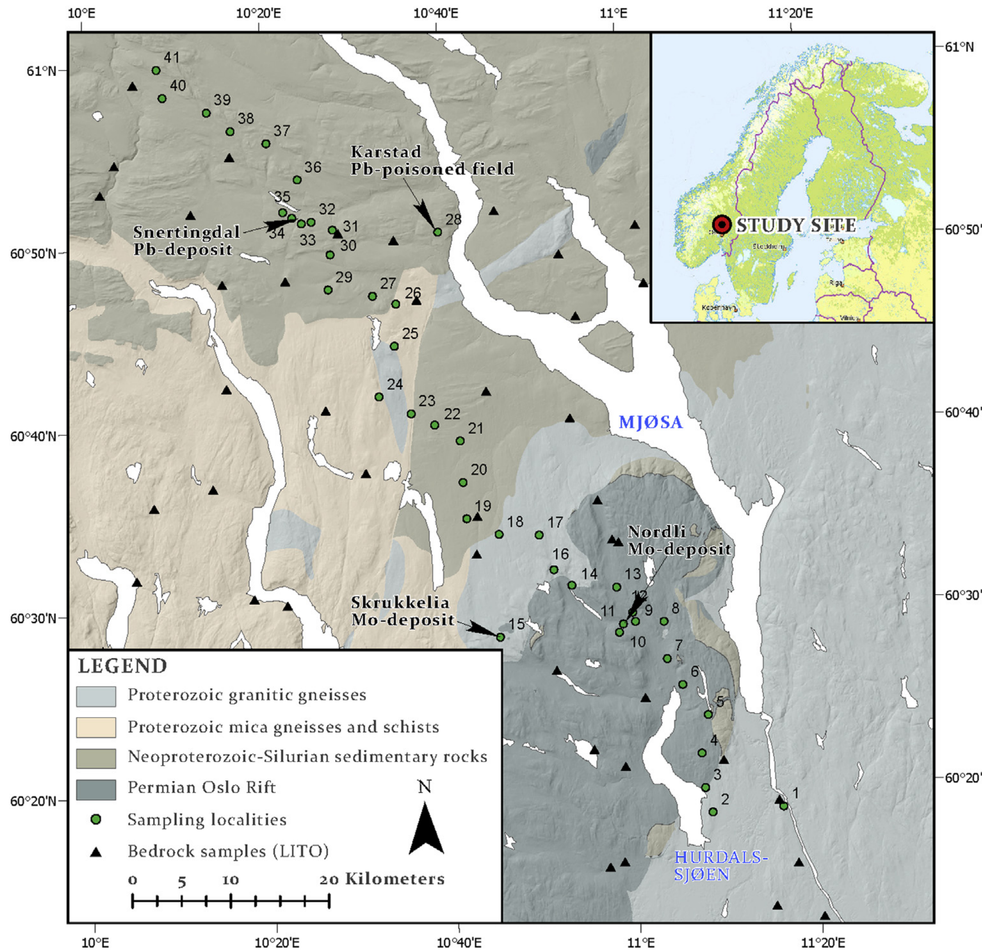

Fig. 1. Bedrock geological map (simplified from Nordgulen, 1999) with sample sites along the Gjøvik transect (Flem et al., 2018). The locations of the Pb-mineralisation at Snertingdal and the Mo mineralisations at Nordli and Skrukkelia are indicated. Specimens of *Lactarius rufus* were not collected at location 7.

Table 1
Analytical results for 40 samples of *Lactarius rufus* collected along the Gjøvik transect, southern Norway. All values in mg/kg. DL: detection limit, MIN: minimum, MED: median and MAX: maximum concentration observed.

Element	DL	MIN	MED	MAX	Element	DL	MIN	MED	MAX
Ag	0.002	0.087	0.72	2.7	Na	10	112	334	1801
Al	100	4.5	16	168	Nb	0.01	< 0.01	< 0.01	< 0.01
As	0.1	< 0.1	0.18	0.45	Ni	0.1	0.23	0.65	1.9
Au	0.0002	< 0.0002	< 0.0002	0.0011	P	10	3661	6005	9012
B	1	< 1	< 1	2.5	Pb	0.01	0.15	0.59	7.9
Ba	0.1	0.1	0.85	5.4	Pd	0.002	< 0.002	< 0.002	0.008
Be	0.1	< 0.1	< 0.1	< 0.1	Pt	0.001	< 0.001	< 0.001	0.0031
Bi	0.02	< 0.02	< 0.02	0.043	Rb	0.1	84	327	1008
Ca	10	42	116	408	Re	0.001	< 0.001	< 0.001	< 0.001
Cd	0.01	0.15	1.08	9.1	S	100	1090	2246	3490
Ce	0.01	< 0.01	< 0.01	0.2	Sb	0.002	< 0.002	0.013	0.448
Co	0.01	0.027	0.19	2.8	Sc	0.1	< 0.1	0.18	0.49
Cr	0.1	1.5	1.9	2.7	Se	0.05	< 0.05	0.18	1.40
Cs	0.005	0.33	2.0	12.1	Sn	0.02	0.024	0.067	0.15
Cu	0.01	17	24	56	Sr	0.5	0.54	0.80	3.9
Fe	10	17	25	56	Ta	0.001	< 0.001	< 0.001	< 0.001
Ga	0.05	< 0.05	< 0.05	0.17	Te	0.02	< 0.02	0.027	0.11
Ge	0.01	0.005	0.085	0.182	Th	0.01	< 0.01	< 0.01	0.022
Hf	0.001	< 0.001	< 0.001	0.004	Ti	1	3.0	7.3	17
Hg	0.001	0.024	0.089	1.13	Tl	0.005	< 0.005	0.025	0.373
In	0.02	< 0.02	< 0.02	< 0.02	U	0.01	< 0.01	< 0.01	0.137
K	100	27783	39241	55305	V	1	< 1	1.2	2.5
La	0.001	< 0.001	0.003	0.077	W	0.1	< 0.1	< 0.1	< 0.1
Li	0.01	< 0.01	0.021	0.27	Y	0.001	< 0.001	< 0.001	0.028
Mg	10	757	1018	1456	Zn	0.1	64	106	273
Mn	1	9	36	372	Zr	0.01	< 0.01	< 0.01	0.14
Mo	0.01	< 0.01	0.023	0.085					

Table 2
Median (MED) values and inter-quartile range (IQR) of 32 elements for the soil O— and C— horizon and *Lactarius rufus* (LAC). All analytical results in mg/kg. For comparison the median for all plant materials collected along the transect is also provided (Reimann et al., 2018). Range represents minimum to maximum concentration. Results from previous mushroom studies only include "wild" samples from uncontaminated sites. Median ratio shows the enrichment and depletion of elements in the collected media.

ELEMENT	O-horizon		C-horizon		All plants	Lactarius rufus this study		Lactarius rufus literature		Mixed mushrooms literature		O-hor/C-hor	Median ratio		
	MED	IQR	MED	IQR	MED (21)	MED	IQR	Range	Reference	Range	Reference		LAC/O-hor	LAC/C-hor	LAC/PLANTS
Ag	0.65	0.69	0.03	0.02	0.004	0.77	0.93			0.07–133	(4,14,21)	23	1.1	25	195
Al	2472	3934	12501	4580	102	16	7.8	10–253	(18,23)	5–2382	(21,23,25,26)	0.2	0.01	0.001	0.16
As	1.6	1.1	4.4	3.1	0.05	0.18	0.18	0.85–1.8	(18)	0.06–182	(1,4,5,14,21,26)	0.4	0.11	0.040	3.6
Ba	147	69	30	18	94	0.85	0.71					4.8	0.005	0.028	0.009
Ca	1951	1730	644	364	4883	116	79	58–70	(20)	25–8800	(13,21,25,26)	3.0	0.06	0.18	0.023
Cd	0.73	0.45	0.11	0.11	0.09	1.08	0.89	0.6–1.68	(18,20,23)	0.08–125	(1,4,5,6,7,10,11,14,15,16,17,19,21,23,24,25)	6.6	1.5	9.8	12
Co	1.9	1.51	5.4	4.0	0.10	0.19	0.25	< 0.02–5.9	(2,18)	0.04–3.3	(1,14,19,21,24,25,26)	0.4	0.10	0.035	1.8
Cr	4.1	3.0	18	7.7	1.8	1.8	0.18	0.2–0.25	(18)	0.08–16	(14,19,21,24,25,26)	0.2	0.5	0.10	1.0
Cs	0.30	0.25	0.91	0.52	0.10	2.0	2.7					0.3	6.6	2.2	21
Cu	8.0	3.7	9.6	7.8	4.6	25	12.7	13–38	(18)	0.14–260	(1,4,5,6,7,10,11,13,14,15,16,17,19,21,24,25,26)	0.8	3.1	2.6	5.4
Fe	3606	4028	21671	8031	75	32	16	34–4757	(2,18,23)	4.6–4660	(1,6,7,10,11,13,14,15,16,17,19,21,23,24,25,26)	0.2	0.01	0.001	0.43
Hg	0.23	0.11	0.03	0.02	0.014	0.089	0.057	0.01–0.13	(18,20)	0.02–22	(1,4,5,6,10,11,14,15,21)	7.3	0.38	2.7	6.6
K	808	265	540	295	5388	39241	12169			12600-76000	(1,13,21,25)	1.5	49	72	7.3
Li	0.39	0.98	15	10.2	0.019	0.02	0.019					0.03	0.05	0.001	1.1
Mg	509	205	2784	1935	1003	1018	194			167–4540	(13,19,21,22,26)	0.2	2.0	0.37	1.1
Mn	148	184	215	172	959	36	21	15–65	(18,23)	1.9–179	(1,5,6,7,10,11,13,14,15,16,17,19,21,23,24,25,26)	0.7	0.25	0.17	0.04
Mo	1.2	0.86	2.5	1.8	0.15	0.023	0.02					0.5	0.02	0.009	0.16
Na	124	32	29	16	24	334	319			12–4850	(1,13,21,26)	4.3	2.7	11	14
Ni	4.4	1.7	14	15	0.91	0.65	0.27	0.48–0.9	(18)	0.25–14.2	(14,19,21,24,25,26)	0.3	0.15	0.047	0.7
P	871	283	269	164	1025	6005	1330					3.2	6.9	22.3	5.9
Pb	62	40	11	5.5	0.33	0.59	0.59	3,2–9,1	(23)	0.05–28	(6,7,10,11,14,16,17,19,23,24,25,26)	5.5	0.01	0.05	1.8
Rb	6.6	3.1	9.0	3.1	19	327	180			5,2-1710	(1,21)	0.7	50	36	17
S	851	413	147	144	716	2246	549					5.8	2.7	15	3.1
Sb	0.90	0.54	0.17	0.13	0.01	0.013	0.033	0,03–0,09	(3)	0.02–12	(1,3,14)	5.4	0.01	0.07	1.2
Sc	0.74	0.38	1.6	0.91	0.16	0.18	0.129			< 0,001–0,16	(12,21)	0.46	0.25	0.11	1.1
Se	0.65	0.52	0.30	0.18	0.10	0.18	0.19	< 0.1–25	(2,18)	0.01–24	(1,4,5,10,11,14,21,26)	2.2	0.28	0.60	1.8
Sn	1.8	1.3	0.30	0.13	0.05	0.067	0.039					6.1	0.04	0.22	1.2

(*continued on next page*)

Table 2 (*continued*)

ELEMENT	O-horizon		C-horizon		All plants	Lactarius rufus this study		Lactarius rufus literature		Mixed mushrooms literature		O-hor/C-hor	Median ratio		
	MED	IQR	MED	IQR	MED (21)	MED	IQR	Range	Reference	Range	Reference		LAC/O-hor	LAC/C-hor	LAC/PLANTS
Sr	23	14	6.0	4.9	18	0.80	0.51			2–14.7	(14,26)	3.8	0.04	0.13	0.045
Ti	68	61	228	166	1.9	7.3	3.4	0.3–0.8	(18)			0.3	0.10	0.03	3.8
Tl	0.13	0.045	0.11	0.06	0.013	0.025	0.028			0.01–0.17	(14,26)	1.2	0.18	0.23	1.9
V	6.2	4.2	27	11.5	1.1	1.2	0.31			0.02–202	(5,10,11,21,26)	0.2	0.19	0.043	1.0
Zn	51	27	40	23	38	106	30	35–516	(2,18,23)	6.6–590	(1,4,5,6,7,10,11,13,14,15,16,17,19,21,23,24,25,26)	1.3	2.0	2.6	2.8

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2.4. Quality control

For laboratory quality control purposes, the laboratory inserted and analysed their own vegetation CRM samples, CDV-1 and V16, approximately three times with each material (approximately 40 samples). In addition, an in-house project mineral soil standard and a project organic soil standard used for other regional geochemical sampling campaigns (Andersson et al., 2015; Finne and Eggen 2013, 2015; Reimann et al., 2012) was included on a regular basis among the samples before they were sent to analysis. Due to the low sample weight of mushrooms at all locations, no duplicates were available for analysis.

2.5. Data analysis

The analysis of geochemical compositions of different media along a transect must take into account the closure effect (Aitchison, 1986) within each medium, and should use a calculation method for trends and correlations that is robust against outliers and scatter. Significant problems with closure occur when studying bivariate plots for element concentrations within a single medium, because each data point - representing one sample - could be shifted along a line $y = a\,x$ through this point by changing the abundance of other elements within this sample. This problem is relatively minor for plant materials as sample media because here the abundance of the main elements is narrowly constraint, but the effect is known to be substantial in rocks and soils (Filzmoser et al., 2010; Reimann et al., 2012). Calculated bivariate relations therefore have to be interpreted as being exploratory and qualitative. They cannot be quantitatively compared if they relate element concentrations within the same sample. For comparing different sample media, bivariate plots can be used as normal.

For studying element correlation within *Lactarius rufus,* the data were thus log-ratio transformed using the method described in Kynčlová et al. (2017). For studying the correlation between soil O and C horizon and *Lactarius rufus* a simple log-transformation is sufficient.

3. Results

Table 1 summarises the analytical program, detection limits and results for *Lactarius rufus* for all 53 elements analysed. In the following text, only those 32 elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, Ti, Tl, V and Zn) for which the majority of the analytical results were above the respective detection limit will be discussed. Results for the soil O— and C-horizon are reported in Flem et al. (2018), while all plants are discussed in Reimann et al. (2018).

Table 2 shows the median concentrations of these 32 elements in the soil C— and O-horizons, *Lactarius rufus* and in all plant materials collected along the transect. As a measure of variation, the robust inter-quartile-range (IQR) is used. For comparison, concentrations for *Lactarius rufus* and other mushroom species from previous studies are also provided. These results are from studies where no account has been taken of the season, weather or age of mushroom stand, all of which could influence the metal uptake (Rudawska and Leski, 2005). The median ratios between the different sample materials are provided to detect enrichment or depletion of elements in *Lactarius rufus*.

Compared to all plants (birch, spruce, cowberry and blueberry leaves and twigs, fern, horsetail and pine bark) collected within this sampling campaign (Reimann et al., 2018), *Lactarius rufus* is variably enriched in many elements: Ag (195 times), Cs (21), Rb (17), Na (14), Cd (12), K (7), Hg (7), P (6), Cu (5), Ti (4), As (4), S (3) and Zn (2.8 times) (Table 2). A similarly strong enrichment of Ag has been noted in several studies (Borovička et al., 2007; Byrne et al., 1979). Elements that are most depleted in *Lactarius rufus* when compared to the median of all plants are Ba, Ca, Mn, Sr, Al and Mo (Table 2).

Compared to the soil O-horizon, *Lactarius rufus* is enriched in: Rb (50 times), K (49), P (7), Cs (7), Cu (3), S (2.7) and Na (2.7). In contrast,

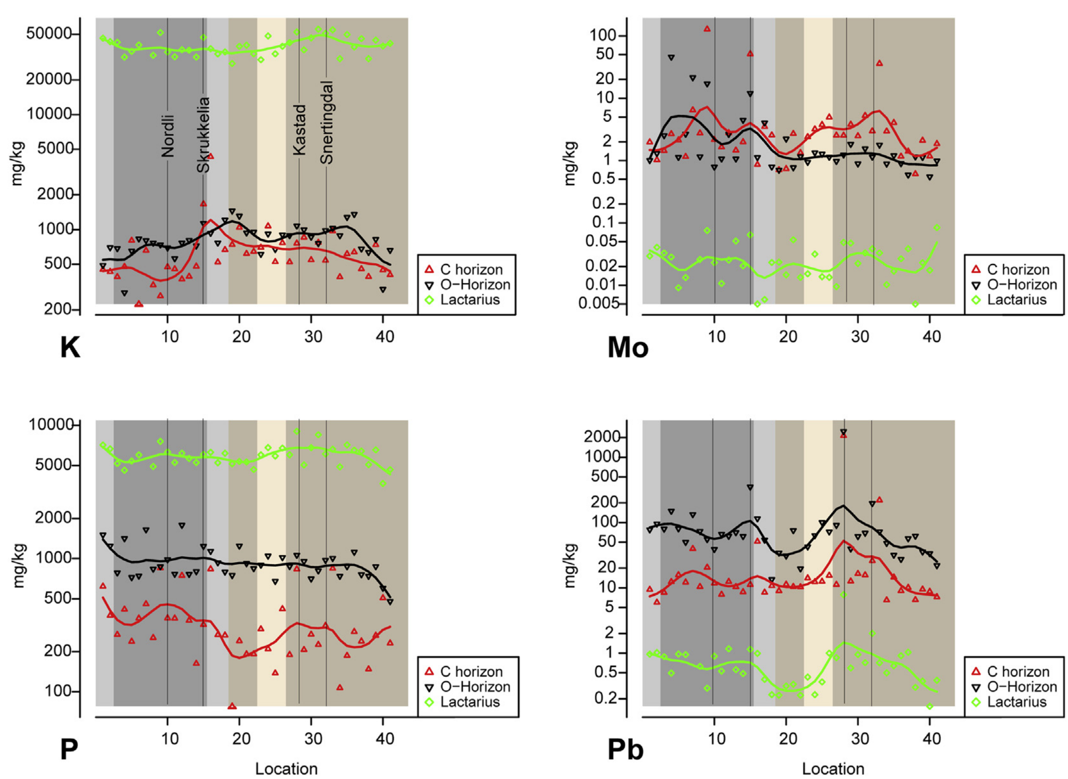

Fig. 2. Transect plots for the substrate and the mushroom. The colored lines show the moving median for the materials considering the two closest sites. Background colours in plots represent the lithology as in Fig. 1. The locations of the main mineralisations and the lead-poisoned area at Kastad encountered along the transect are marked by vertical lines and identified in the potassium plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

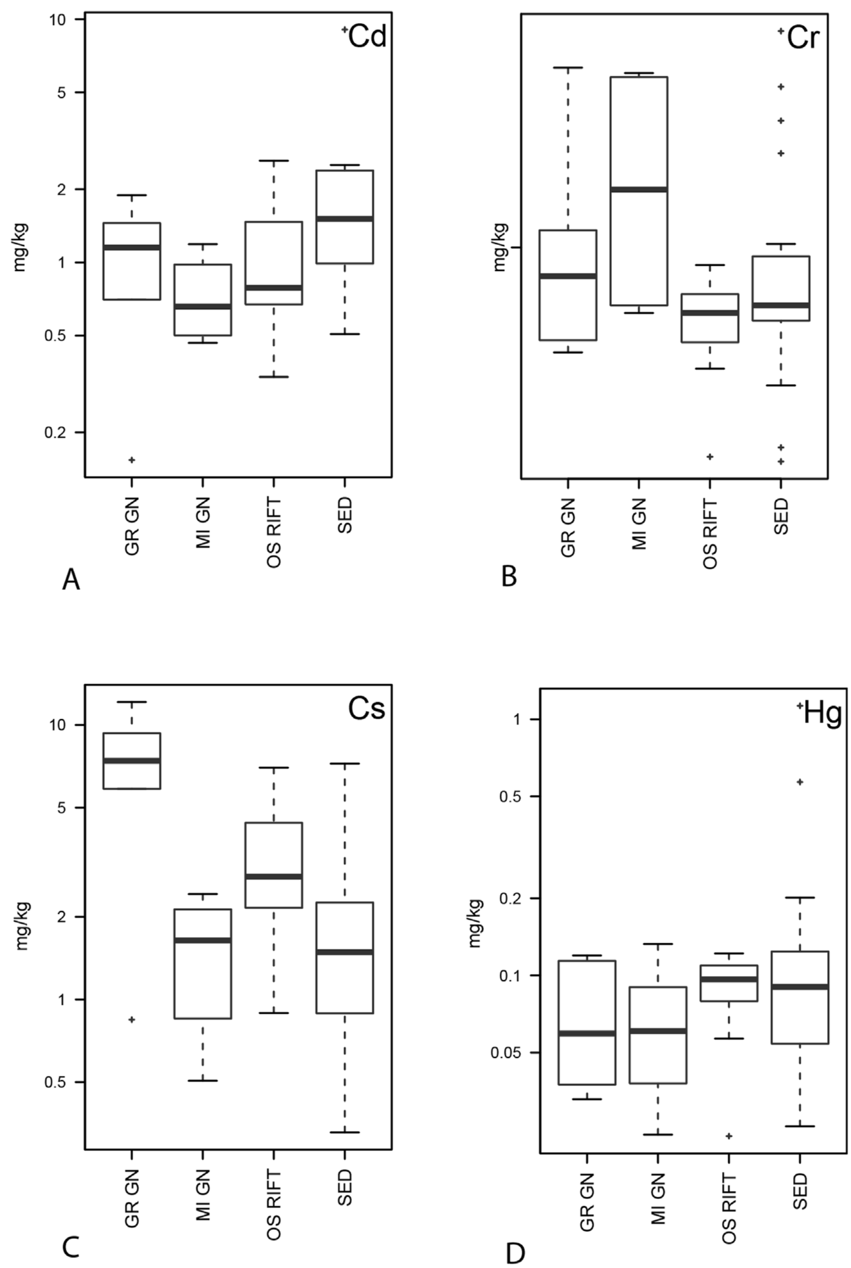

Fig. 3. Boxplots providing a comparison of element concentrations of Cd, Cr, Cs and Hg in *Lactarius rufus* depending on the lithology under the substrate.

uptake of Ba, Al, Fe, Pb and Sb is strongly restricted, which is also noted by Borovička et al. (2006) regarding Sb. (Table 2).

Compared to the soil C-horizon *Lactarius rufus* is enriched in K (72 times), Rb (36), Ag (25), P (22), S (15), Na (11), Cd (10), Hg (3) and Cu (2.6). The concentration of Li, Al, Fe and Mo is most strongly depleted compared to the C-horizon (Table 2). Table 2 also displays that the O-horizon is enriched in Ag, Cd, K, Na and Hg compared to the C-horizon. This enrichment could indicate that the mushroom might be a contributing factor to the up concentration of these elements in the O-horizon.

The mushrooms in this study show similar concentrations as those from previous studies (Allen and Steinnes, 1978; Borovička et al., 2006, 2007; Byrne et al., 1979; Byrne and Ravnik, 1976; Chojnacka and Falandysz, 2007; Chudzyński and Falandysz, 2008; Falandysz and Chwir, 1997, 2004, 2007, 2008, 2017; Genç;celep et al., 2009; Kalač and Svoboda, 2000; Kojo and Lodenius, 1989; Kowalewska et al., 2007; Malinowska et al., 2004; Moilanen et al., 2006; Ouzouni et al., 2007; Perkiömäki et al., 2003; Řanda and Kučera, 2004; Rudawska and Leski, 2005; Sarikurkcu et al., 2010; Sarikurkcu et al., 2012; Tel-Cayan et al., 2017), with the exception of Sc, which shows higher concentrations in this study, and Sr, which shows lower concentrations. This study shows lower concentrations of As, Fe, Pb and Sb in *Lactarius rufus*, while the Ca, Cr and Ti levels are much higher than in previous studies of the same fungus (Borovička et al., 2006, 2007; Moilanen et al., 2006; Perkiömäki et al., 2003; Rudawska and Leski, 2005).

4. Discussion

4.1. Lead and molybdenum mineralisations

Most elements do not show clear concentration trends along the transect but rather an indistinct variation in element concentrations from one location to the next. The Kastad lead-poisoned area near Gjøvik (location 28 in Fig. 1) is indicated by an approximately 8 times higher Pb concentration in *Lactarius rufus* than the median concentration along the remainder of the transect (Fig. 2).

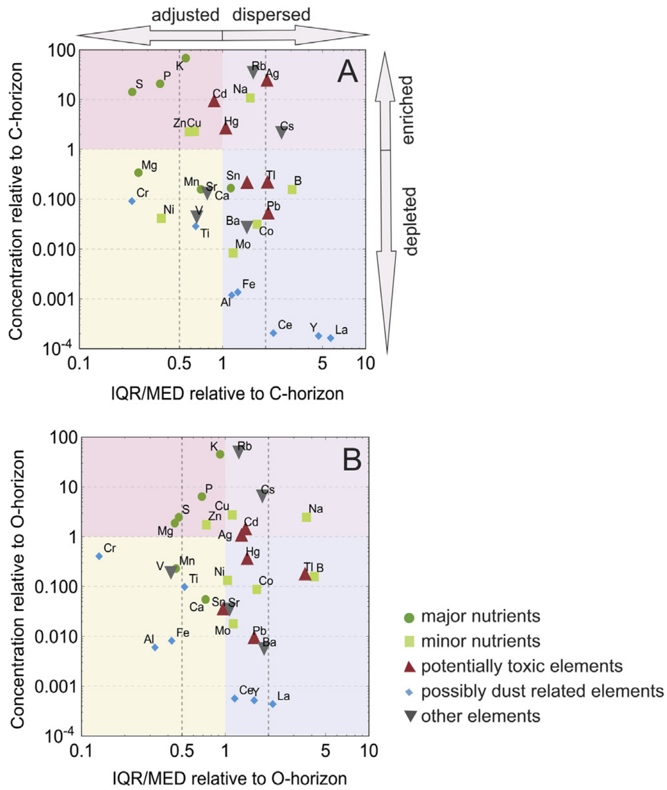

Fig. 4. Element concentration and variation in *Lactarius rufus* normalised to the substrate: O-horizon (A) and C-horizon (B).

This location shows lead concentrations of more than 2000 mg/kg in the two soil horizons (Flem et al., 2018). Snertingdal, the other Pb-occurrence within the transect also shows a higher Pb-concentration in the soil horizons, but is not reflected in the mushrooms. According to Låg and Bølviken (1974), the Kastad location lies in a topographic low point where lead-bearing groundwater emerges at the surface of the poisoned area. That, and higher Pb-concentrations in both soil horizons, may explain why the Pb-concentrations are remarkably higher on the Kastad location. The Mo− mineral occurrences in the Oslo Rift are also marked by anomalies in the soil O and/or C horizon (see Flem et al., 2018) but these high Mo concentrations in the substrate are not reflected in the mushrooms.

It appears that *Lactarius rufus* shows the Snertingdal lead mineralisation and the Kastad lead-poisoning field (location 28 in Fig. 1), as there is an enrichment of Ca, K, Na, P, Sb, Se, Sr, U and Zn compared to the rest of the transect. It appears that *Lactarius rufus* reacts with increased uptake of these elements, including two major nutrients, as a reaction to the high, toxic lead concentrations. For other elements along the transect, it is unclear why there are extreme values that only occur at single sites.

4.2. Lithology effects

Boxplots are here used to study the differences in element uptake from soil formed on the different lithologies. Some differences that are related to lithology can be observed. Soils on the mica gneiss and schist generally show lower concentrations compared to the other lithologies, except for the elements Cr and Sc. The highest concentrations of Ag, Al, As, Ba, Ca, Cd, Co, Cu, Fe, K, Li, Mg, Na, Ni, S, Sb, Sr, U and Zn in *Lactarius rufus* are observed on the Neoproterozoic sedimentary rocks. This stands in contrast to the analytical results for the bedrock samples (LITO samples in Fig. 1) where the elements Al, Ba, Ca, Fe, K, Na, Ni, U and Zn generally show the lowest concentrations within these

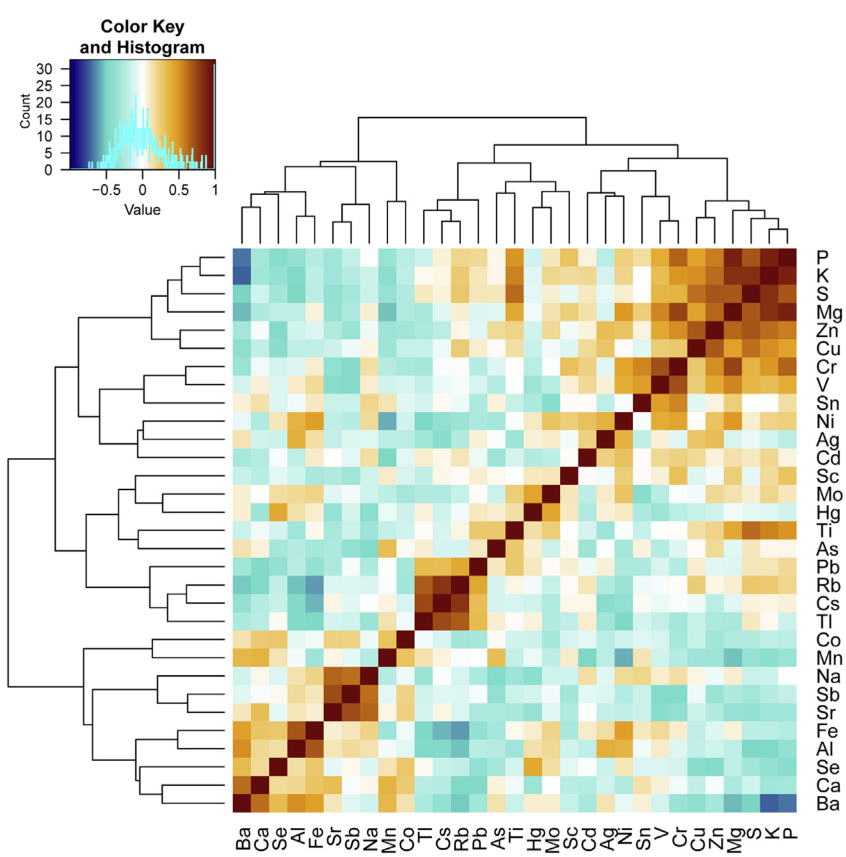

Fig. 5. Heat map showing the correlation between elements within *Lactarius rufus*.

sedimentary rocks.

Mushrooms on granitic gneisses show considerably higher concentrations of Cs, Rb and Tl than those on other lithologies. Only Hg and Te show the highest concentrations on the Permian intrusives of the Oslo Rift (see Fig. 3 for examples).

4.3. Enrichment/depletion and variation

The relative coefficient of variation (IQR/MED) can be used to detect changes in the element variation when comparing sampling media. Fig. 4 summarises element concentrations and variations in *Lactarius rufus* relative to the O– and C-horizon of the substrate. The elements are divided into five groups: major and minor nutrients, potentially toxic elements, elements that might be related to dust and the remaining elements. The figure shows the relative enrichment/depletion of elements in *Lactarius rufus* compared to the two soil horizons combined with the variation change or spread for all elements.

Some elements show, in addition to enrichment in mushrooms in relation to the C– (Fig. 4A) and O-horizon (Fig. 4B), a clear regulation of uptake into a preferred concentration range (shown as significantly decreased variation in *Lactarius rufus*). The major nutrients K, P, S and Mg are enriched in *Lactarius rufus* and the fungus appears to control the uptake to a preferred concentration range. This is also evident for Cu and Zn, which was also seen by Rudawska and Leski (2005). The high uptake of K in mushrooms in Norway is also documented by Allen and Steinnes (1978). The major nutrients Mn and Ca, on the other hand, are depleted when compared to the substrate (both C– and O-horizon).

An especially low variation in *Lactarius rufus* is shown by the nutrients Mg, P, K, S and Zn (see Table 2 and Fig. 4), followed by the elements Cr and V. The concentration of nutrients thus appears to be especially well regulated in the mushroom. In contrast, the elements Sb, Ag, Co, Te, Li, Tl, Cs and Pb, most of them potentially toxic elements, show a high variation in *Lactarius rufus* (Table 2).

4.4. Correlation with substrate and within Lactarius rufus

Element concentrations in *Lactarius rufus* and its substrate, the soil O– and C-horizon, display poor to non-existent correlation. In general, *Lactarius rufus* appears to take up whatever it needs, independent of the concentration in its substrate. It also appears that the mushroom is able to utilize the element pool in both organic and mineral soil.

To study element correlations within the mushroom a correlation analysis, which is well suited for compositional data, was used here (Kynkova et al., 2017; Reimann et al., 2017). This combines correlation analysis of centered log ratio-transformed data with a cluster analysis. Fig. 5 shows the correlation of 31 of the 32 considered elements within *Lactarius rufus*. Lithium was removed due to a relatively high number of values below detection. The diagram demonstrates a high correlation between the major and some minor nutrients (P, K, S, M, Zn, Cu – upper right corner). At the other end of the heat map (lower left corner) Ba, Ca, Se, Al and Fe show rather good correlation – the group of elements that are strongly depleted in the mushroom when compared to all other plants. Some further interesting element clusters occur and are visible along the vertical line in the diagram, e.g., Sr, Sb and Na or Cs, Rb, Pb and As. Titanium also correlates reasonably well with the major nutrients Mg, K, S, P. Reimann et al. (2015) provided data that suggest that Ti may play an underestimated role in plant nutrition. The diagram can thus be used to develop ideas about the uptake mechanisms and routes of these elements into the mushroom.

5. Conclusion

The data suggest that the fungal element turnover, consisting of selective enrichment and interchange via mycorrhiza as well as heavy metal exclusion or detoxification, is not yet fully understood. What can be seen, however, is the selective enrichment of nutrient elements and a selective exclusion or enrichment of single elements.

Compared to all the plants collected along the transect, *Lactarius rufus* is enriched in many elements, most impressively in Ag but also in a number of potentially toxic elements such as Cd, As, Hg and Cu. Compared to the two soil horizons, the enrichment of the major nutrients K, P and S, together with Rb in *Lactarius rufus* is substantial. In contrast, the major nutrient Ca is only taken up to a very limited amount in *Lactarius rufus* and depleted when compared to the median of all plants.

Lactarius rufus, though accumulating many metals, is not a suitable sample medium for biogeochemial exploration for porphyry molybdenum or sandstone-hosted lead deposits under Norwegian field conditions, as high concentrations of these elements in the substrate are not reflected in the mushroom.

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