METALS UPTAKE IN PINE NEEDLES *(PINUS NIGRA)*

Abstract

The current study investigated the use of Pine trees *(Pinus nigra)* as bio-indicators. Metal uptake and accumulation is determined at three sites, which vary in traffic volume. The metals that are in focus in this study are Ca, K, Mg, Na, Sr, Ag, Pb, U, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, and As. First, the obtained data were checked for statistically significant difference within the sampling period May to August, but no trend was found. Bioconcentration factors were calculated to indicate metals accumulation and bioavailability. The accumulation of some metals demonstrate that pine trees can be used as bio-indicators.

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Introduction

Metals in soil and plants

Soil is an essential factor in ecological cycles where it has an important role in benefiting the ecosystem services. Such services help in breaking down the environmental contaminants by acting as a water filter. All ecosystems include varieties of life, but soil even includes a greater variety of life than other ecosystems. Soil contains a lot of different organisms which have a main role in the growth of plants. In addition to that, the presence of several antibiotics in soil which helps to fight diseases. People need soil to facilitate preserving their solid waste where they use soil as a space for these wastes. People also need soil as a filter for sewage and its role in purification services. Soil can be considered as a foundation for today's cities and is part of a successful agriculture. Therefore, soil is essential in the agroecosystem, which supply humans with food and other materials [1].

Metals are defined as elements that show metallic characteristics such as conductivity, malleability, ductility, ligand specificity, and cation stability. The so called heavy metals are defined to have high density at least 5 g cm^{-3}, a high atomic weight, as well as an atomic number that is greater than 20 [2]. From environmental interest are elements, that can pose risk to plants, animals and/or human, so called potentially toxic elements (PTE). They can be bound to silicate minerals and represent in this covalent bond the background soil metal concentration, whereby these bound metals do not cause pollutions as those that are found in separate entities [3]. Certain metals are listed as some of the important contaminants in environment. For example, mercury and lead are listed in the World Health Organization's list of ten chemicals of major concern as they are considered as dangerous elements due to their high-water solubility and toxicity [4]. The existence of such metals in soil is a natural thing. They are necessary for plants, but it is their high concentration in soil, which is harmful. There are some factors that affect the content and the behavior of these metals in soil such as the geological and human activities. These activities affect the concentration of heavy metals by increasing it to larger levels where harmful effects to plants as well as animals may occur. This may also result in a disturbance in the function of the ecosystems. Some examples of these activities are the use of fertilizers and pesticides, the burning of fossil fuel, mining, getting rid of municipal waste and industries that produce mineral products [5], [6], The geological and human activities can be sources for heavy metals, which may be accumulated in the human body for a long time. For example, Cd is considered to have a 10-year half-life in humans [7]. Cadmium is a lustrous, ductile, silver-white, malleable metal. Cadmium is a soft metal where it can be cut with a knife and it tarnishes in air. It is soluble in acids but not in alkalis. In addition, in the presence of oxygen it readily forms highly water soluble complexes. High levels of cadmium are harmful for human health where it may cause damage to the central nervous system, the immune system, DNA damage, and may also be factor of cancer development [8].

Wastewater may also contain potentially toxic elements such as Cu, Pb, Zn, Cd, Ni, Cr, Mn, Hg [9]. The possibility of having accumulated potentially toxic elements in agricultural ecosystems, is very high due to using wastewater in irrigation for long periods. [10], [11]. However, Fe, Ca and Mg are also metals, but they are considered of vital importance to the nutritional and medical needs, which are recommended as daily amounts, of human beings. At the same time, there are other metals, which are known to be toxic even in low concentrations such as As, Pb, and Cd. These toxic metals do not seem to have any biological importance in biochemistry or physiology [12].

Some of the effects that potentially toxic elements have on soil can be seen for example for lead. Soil productivity is negatively influenced by high concentrations of lead (more than 1000 mg/kg) while lower concentrations (less than 400 mg/kg) may inhibit necessary plant processes such as: water absorption with toxic symptoms of dark green leaves, photosynthesis, withering old leaves, stunted foliage with short brown leaves and stunted foliage with short roots [13].

Metals in pine needles

Plants use different elements as structural parts in carbohydrates and proteins, organic jots in metabolism like magnesium in chlorophyll and phosphorus in ATP, an enzyme activator such as potassium, and for preserving osmotic balance [14]. The metal content of needles is affected by root uptake from soil where it contributes to the metal content of needles. Pine needles are known to be bioindicators, but their influence differs according to their species and growing area [15]. The uptake of elements can happen by different ways such as the roots uptake from soil or in leaves taken from air by deposition of both dry and dissolved metal species. Trace elements can also be taken up by these ways [16]. The needle content of metals depends on the age of the pine shoot where a significant difference can be noted between one-year old shoots and older ones [17].

Pine trees are the most common coniferous tree that cover vast areas around the world [18]. There are approximately 115 trees and shrubs species of the pine genus [19]. Most of the pine trees live in the northern hemisphere [20], where they can be found in natural forests as well as botanical gardens [19]. Pine tree species are known to have high uptake of arsenic in polluted soils. They are usually used in treating arsenic and soil polluted by metals, because they are noted to have strong resistance to arsenic and other potentially toxic elements [18]. Here are some of the common species of Pinus:

Pinus sylvestris, Pinus virginiana, Pinus contorta, Pinus lambertiana, Pinus palustris, Pinus radiata, Pinus edulis, Pinus echinata, Pinus strobus, Pinus banksiana, Pinus ponderosa, Pinus rigida, Pinus albicaulis, Pinus resinosa, Pinus aristata, Pinus coulteri, Pinus taeda, Pinus elliottii, Pinus sabiniana [21].

Pines are evergreen conifers, which have leaves in the form of needles, keeping their needles year-round [22]. Due to the wide spread of pines all over the world and the high possibilities of taking samples, pine needles are thus used as bioindicators. Conifers, unlike deciduous trees, are better suited to be used for environmental quality monitoring since they are subjected to long term exposure [23]. According to Augusto et al [24], conifers absorb more airborne components than deciduous trees due to the waxy surface and large area of the pine needles. Therefore, the conifers absorb about 35% from air while deciduous tree species absorb about 25% [24].

Conifers are the commonly most used high trophic plants for biomonitoring in different areas, industrial and urban, due to their long-term life. Therefore, samples can be used in different investigations over time [22]. It is usually approved to use biological materials in monitoring environmental quality because it is considered a reliable and non-expensive way of getting information about heavy metals pollution. The main reason for that is the ability to avoid expensive costs in cases of long-term comparisons [25]. It is common to use plants as bio monitors in different countries where they are usually used in studying of air pollutants. The samples are widely chosen from mosses in monitoring the precipitation of heavy metals [26]. However, many studies started to use samples of vascular plant leaves such as conifer needles in monitoring of heavy metals [27].

This study focuses on several metals and elements (Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Ag, Cd, Pb and U) in pine needles as well as in the soil where they grow. The purpose of the study is to investigate the accumulation pine trees in the studied area. This was done by calculating the bioconcentration factors for the above-mentioned elements.

Materials and method

Samples:

The samples, which are used in this study, come from both pine needles and soil where they grew. Needle samples are collected from pine trees (*Pinus nigra*) in Vienna (the capital of Austria), The needles were taken from the sunlit part of the crown, pooled sample per tree, 30-50 needles per tree à one pooled sample; fresh samples were not weighed. Soil samples were taken from the place where the pine trees grew, the same three places. Soil samples were taken from the A-horizon using metal free device (small shovel), stones, roots and other bigger particles were removed by hand. Samples were collected during a period of four months, that is, May to August in the year 2015. It can be noted from the table below (table 1) that these samples were collected once every week during this period. The sampling sites were chosen according to the volume in traffic, representing high, medium and low traffic volume (see figure 1). Fresh needles (from 2015) as well as one-year old needles (shoots from 2014) were sampled, For BF calculation only the 1-year old needles were used. Age of needles can range from 3.7 years.

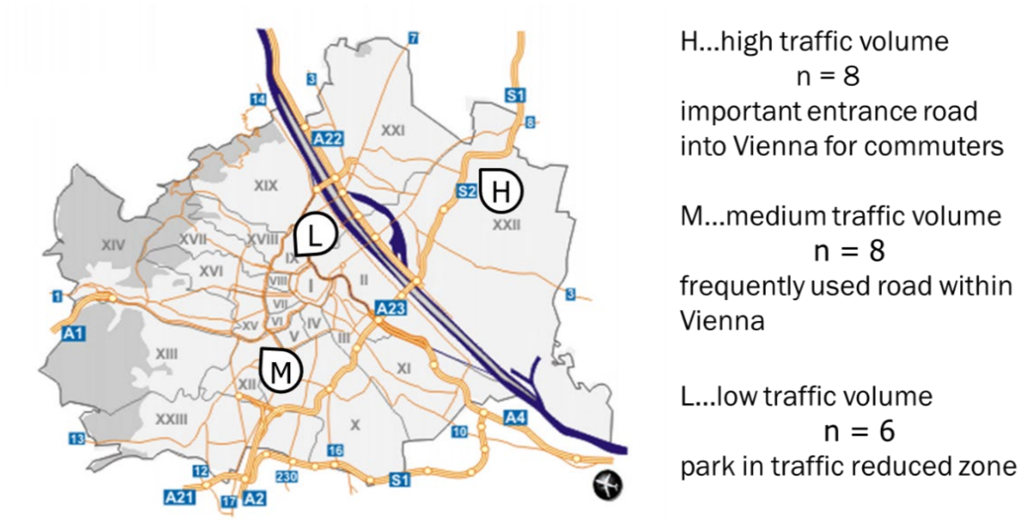


Figure 1: graph sampling sites in Vienna, Austria

Traffic volume:

No traffic count at the roads at sampling sites is available why those in the vicinity were used. Traffic counts are performed only on highways (performed every month: [28]) and municipal road classified as main roads categories A and B (every 5 years: [29]).

Data for H: Daily number of vehicles on municipal road at 1.5 km linear distance from sampling site ~17000. Daily number of vehicles on the highway close to sampling site in Mai 2015 ~43000; June 2015 ~46000; July 2015 ~50000; August 2015 ~49000. () (approx. 4 km linear distance from sampling site) [29]

Data for M and L can only be estimated, since the traffic count includes main roads, but not minor roads or (more or less) traffic free areas

M: close to a crossing of a short minor road one way with only one lane; no permanent through traffic and a two-way road (two lanes). The parallel road of the former (separated by one block of houses), also one way but with 3 lanes, counting point on that road 2.4 km more into the city (where only 2 lanes are left) showed 3924 vehicles per day in 2015 → estimation of 8000 to 10000 vehicles per day.

L: sampling site within a park, no regular traffic permitted.

Sample preparation and measurements

Needle samples were first dried at 105°C, homogenized in a metal free mortar (agate mortar) and stored in small PE bags at room temperature prior to analysis. Before homogenization, the samples were washed in order to avoid any metals attached by air borne deposition This was done by rinsing the needles. After that, the samples were dried at 105°C and grounded into a fine powder and treated in a microwave digestive system (MLS-1200 MEGA) to produce a clear digest solution. Digestion was done in a mixture of 5 mL subboiled nitric acid (HNO_3; Merck, Darmstadt, Germany) and 1 mL hydrogen peroxide (H_2O_2; Merck, Darmstadt, Germany; 30% *w*/*w*). The following heating programme was applied (time in min/power in W):

3/250; 1/0; 4.5/250; 6/650; 5/400; ventilation 25 min. The final volume of 10 mL was produced by adding deionized water (triple distilled, in house production). Prior to measurement by ICP-MS the samples were diluted 1:20 with 1% HNO_3.

Soil samples were dried at 105◦C, homogenized in a metal free mortar and stored in plastic bags at room temperature, awaiting further treatment. Approximately 150 mg per aliquot digested in microwave oven (MLS-1200 MEGA) using a mixture of 1.5 mL subboiled nitric acid and 4.5 mL ultra-pure hydrochloric acid (Merck Darmstadt Germany). The final volume of 10 mL was produced by adding 1% nitric acid. Prior to measurement by ICP-MS the samples were diluted 1:100 with 1% HNO_3.

Instrumental settings:

The methods that were used for measurements were inductively coupled plasma atomic emission spectrometry (ICP-AES) for the major elements, and inductively coupled plasma mass spectrometry (ICP-MS) for all trace elements in this study. Thus, the metal content defined by the treatment for the soil and pine needles were obtained.

Table 1. Instrumental conditions and figures of merit for both analytical methods used

Parameter	**ICP-AES**	**ICP-SFMS**
Instrument	Prodigy High Dispersive ICP-AES (Teledyne Leeman, Hudson, NH, USA)	Element 2 ICP-SFMS (Thermo Fisher; Bremen, Germany)
Output power	1100 W	1300 W
Argon flow	Coolant:18 L min^{-1} Auxiliary: 0.8 L min^{-1} Nebuliser: 1 L min^{-1}	Coolant:16 L min^{-1} Auxiliary: 0.86 L min^{-1} Nebuliser: 1.06 L min^{-1}
Sample flow	1.0 mL min^{-1}	100 µL min^{-1}
Nebuliser	Pneumatic (glass concentric)	PFA microflow
Spray chamber	Glass cyclonic	PC^3 cyclonic quartz chamber
Plasma viewing	Axial	-------
Analytes: wavelengths or *m*/*z* ratio	Ca 317.933 nm Ca 422.673 nm K 766.491 nm Mg 285.213 nm Na 589.592 nm	Li 7(LR*) Be 9 (LR) B 10 (LR) Al 27 (MR) V 51 (MR)

		Cr 52 (MR) Mn 55 (MR) Fe 56 (MR) Co 59 (MR) Ni 60 (MR) Cu 65 (MR) Zn 66 (MR) As 75 (HR) Se 77 (HR) Sr 88 (LR) Mo 95 and 98 (MR) Ag 107 and 109 (LR + MR) Cd 111 (MR) Ba 138 (LR) Pb 208 (LR + MR) U 238 (LR)

* HR – high resolution; MR – medium resolution; LR – low resolution with the nominal mass resolutions being 350, 4500 and 10000

Data treatment

The pine needle digests were measured repeatedly as basis for precision. The day-to-day repeatability was calculated based on analysis of selected samples (n = 3) on different days and was determined to be less than 2%. The analytical test used to check the time trend is Neumann trend test based on 95% probability [30].

The CRMs used were: SRM1575a - Trace Elements in Pine Needles, Eurosoil 7 and BCR 600 for soil.

Results and discussion

As the samples were collected every week so Neumann test was used to check any significant time trend between the samples from each site for soil, as well as needles. (See appendix for data).

The time trends for both major and minor elements were checked using Neumann trend test. A level of significance of 95% was used for decision making in all statistical tests performed. According to Neumann test, no statistically significant time trend is observed for Ca, Mg, and Na at the three sites, but K in the L-samples is statistically significant. Similar results was found for some minor elements in soil where zinc in H samples as well as manganese and molybdenum in L-samples are statistically significant. In statistical trend tests, the p-value represents an estimation of rejecting the null hypothesis (no trend in data) in favor of the hypothesis of having trend in data. Thus, the statistical test is a form of balance between the possible false positives versus false negatives. Therefore, a lower threshold (where the p value is higher) may give a weak signal that falsely refer to a real trend while there is no trend [31]. Therefore, the significant trend which was found for K, Zn, Mo, and Mn was considered as statistically non-significant. The data were blank corrected, dilution factor and original mass of sample was considered to convert to measured data from the instrument (mass concentration in µg/L) into mass content in mg/kg or µg/g.

Bioconcentration factor BF

The bioconcentration factor (BF) is a ratio that can be calculated from contents in plant and soil (pine needles in this study), using the equation below:

Bioconcentration factor (BCF) = Content of metals in plants parts (needles) / Contents of metal in test soil (mg/kg).

Data were not checked for normal distribution, in general environmental data are not normally distributed, especially when n is quite low. But many statistical tests have been proven to be robust and can be applied also for non-normally distributed data

The BF is calculated in this study to check if the pine trees accumulate contaminants . Thus, BF was calculated by dividing the content of a certain element in pine needles by the content of same element in soil. The samples of soil and needles did not show a significant time trend, therefore the minimum, max and mean value were calculated for each element and thereby the minimum, max and mean BF. For that purpose, minimum, maximum and mean metal content were found, and the BF values were calculated in the following way:

Minimum BF value = minimum content of needles / maximum content of soil.

Maximum BF value = maximum content of needles / minimum content of soil.

Mean BF value = mean content of needles / mean content of soil.

The bioaccumulation factors of major elements for the investigated samples are presented in table 2:

Table 2: Bioconcentration factors for major elements

M	K	Ca	Mg	Na
min BF	106	0.0205	0.0593	0.390
max BF	1398	0.249	0.246	22.2
mean BF	278	0.126	0.118	1.28

L		Ca	Mg	Na
min BF	35.17	0.0291	0.0476	0.241
max BF	309	0.416	0.345	3.57
mean BF	103	0.152	0.150	0.736

H		Ca	Mg	Na
min BF	24.73	0.0389	0.0529	0.152
max BF	377	0.487	0.344	1.03
mean BF	78.9	0.128	0.135	0.366

It can be noted that the mean BFs for calcium and magnesium were less than 1 at the three sites while potassium and sodium ranged between low values (min BF) to high levels which are >1 in the three sites. The highest BF value for calcium 0.0487 was at site H and for magnesium 0.345, which could be noted at site L and H. Both values were less than 1 (level 95%) which means that these elements were not allocated to the pine needles. The BF values for sodium ranged from 0.152-1.03 at site H, 0.241-3.57 at site L and 0.390-22.2 at site M. The values for potassium at sites H and L ranged from 25 to almost 400. Potassium at site M ranged from high values to higher ones where its values were always more than 100 and reached very high levels 1400. Most metals have usually a BF value that is less than 1.00 and higher values means that bioaccumulation of the metals will occur [32]. The values for potassium and sodium at all sites are considered high values when compared with a highest value of 1.00 which is the normal value for metals. This means that sodium and potassium were highly bioaccumulated in the pine needles, especially at site M [33]. Potassium showed the highest bio -accumulation at site M but also at sites L and H with at lower values. Sodium was also mostly accumulated at site M but to a lesser extent than potassium. The following figures 2,3,4 show the differences between these elements where it can be easily noticed which elements that have higher BF values in every site:

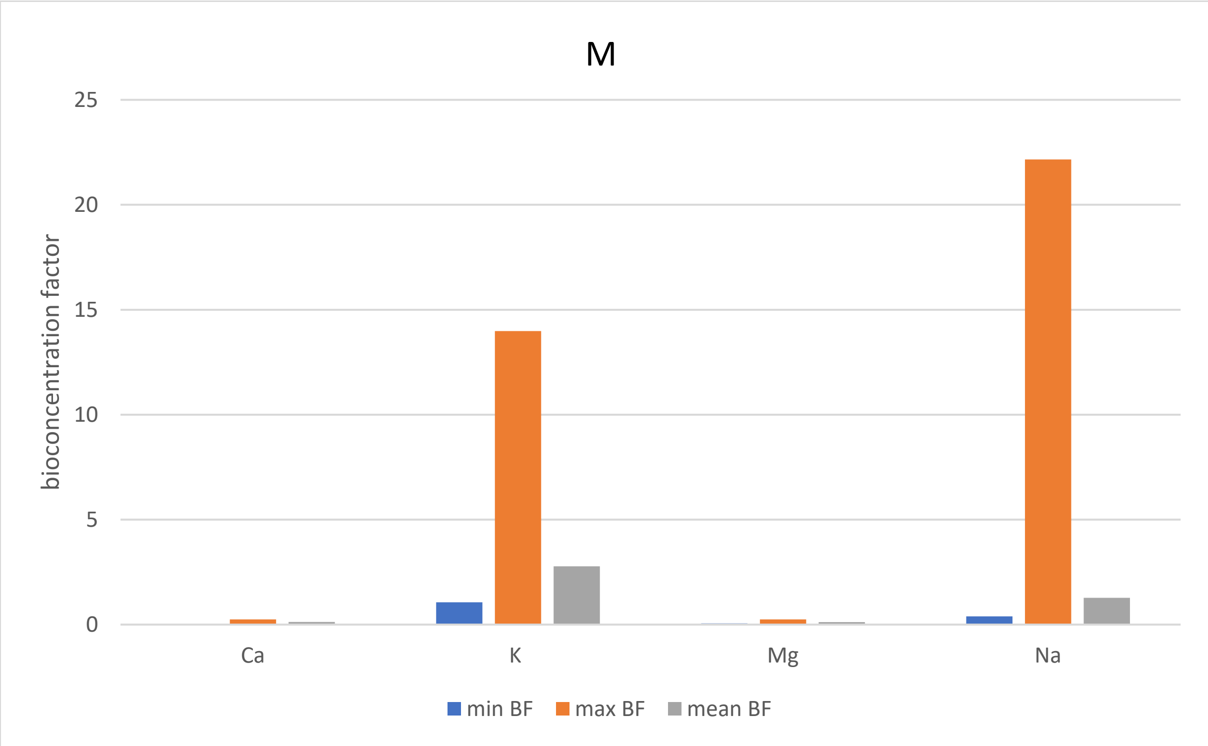


Fig. 2. Bio-concentration factor (BF) for major elements at site M. Values for K are divided by 100.

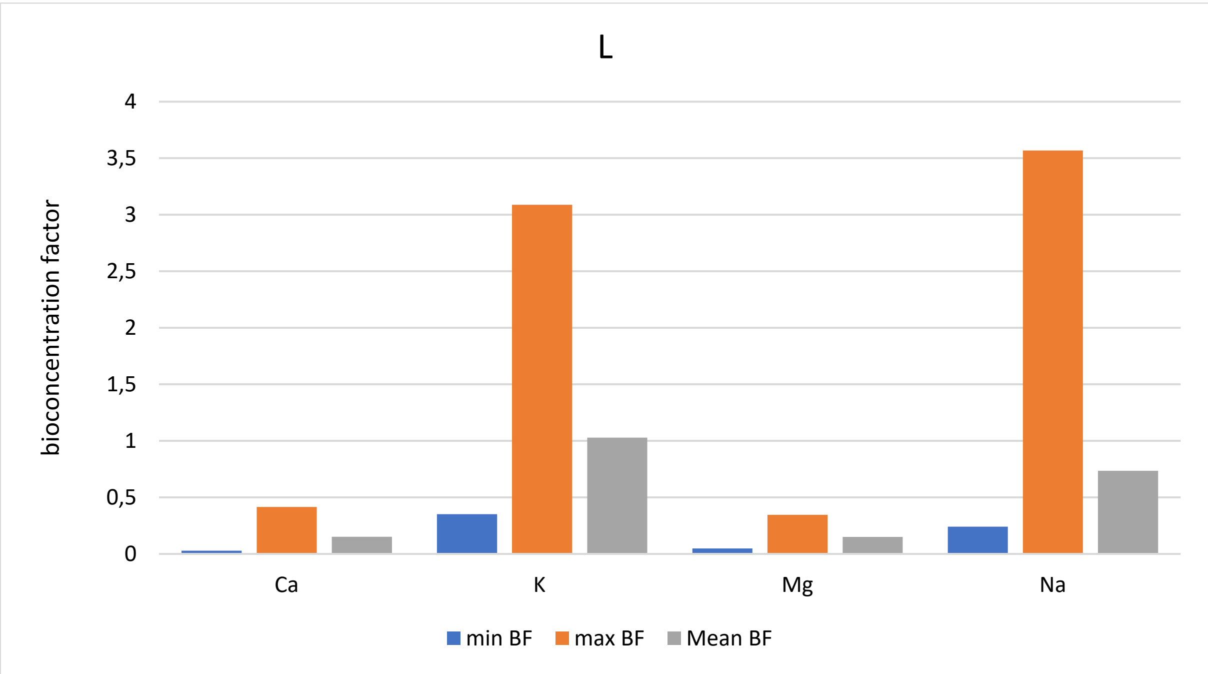


Fig. 3. Bio-concentration factor (BF) for major elements in site L . Values for K are divided by 100.

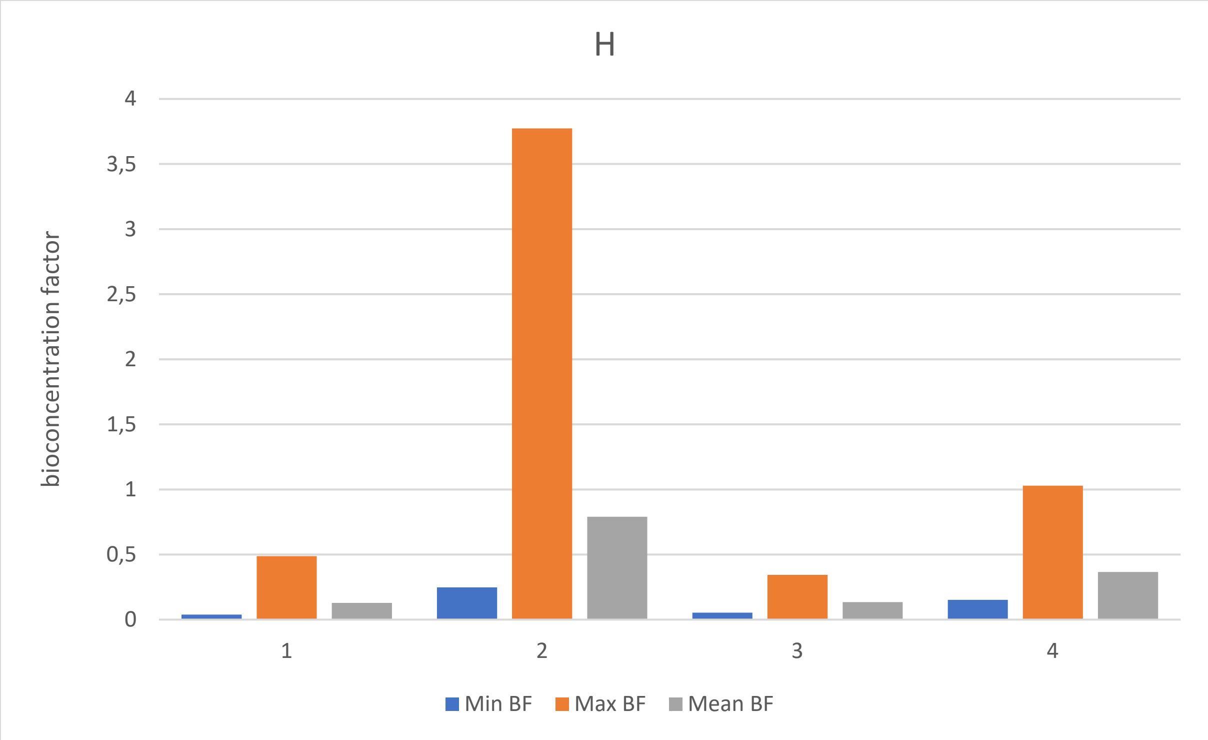


Fig. 4. Bio-concentration factor (BF) for major elements in site H. Values for K are divided by 100.

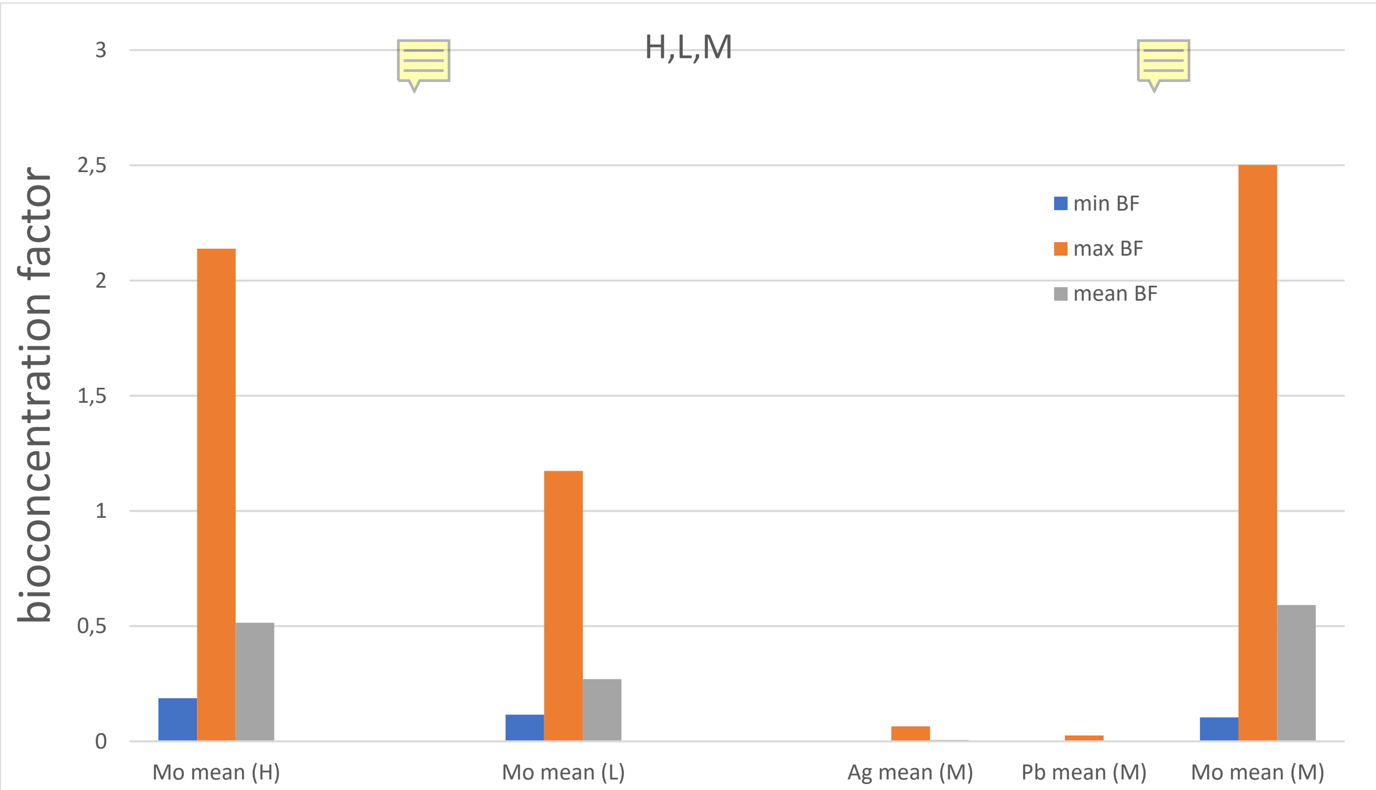
The bioaccumulation factors of minor/trace elements for the investigated samples are presented in table 3:

Table 3: Bioconcentration factors for minor/trace elements

	Site H				Site L				Site M			
Element	**min BF**	**max BF**	**mean BF**	**STDEV BF**	**min BF**	**max BF**	**mean BF**	**STDEV BF**	**min BF**	**max BF**	**mean BF**	**STDEV BF**
Sr	0.0279	0.296	0.137	0.547	0.0151	0.208	0.0833	0.188	0.0302	0.220	0.123	0.344
U	0.000997	0.0254	0.0133	0.0394	0.000563	0.0293	0.00857	0.0438	0.00101	0.0334	0.0104	0.0694
Al	0.00226	0.0234	0.0101	0.0491	0.00155	0.0192	0.00615	0.0459	0.00235	0.0120	0.00583	0.0164
V	0.000418	0.0198	0.00914	0.0545	0.000150	0.0195	0.00540	0.0563	0.0000875	0.0156	0.00544	0.0352
Cr	0.000864	0.0726	0.0257	0.179	0.000917	0.0518	0.0171	0.0503	0.000889	0.117	0.0226	0.0563
Mn	0.0168	0.194	0.0696	0.347	0.0118	0.122	0.0423	0.232	0.0181	0.168	0.0477	0.200
Fe	0.00143	0.0448	0.0183	0.176	0.00134	0.0360	0.0115	0.0121	0.000351	0.600	0.0101	0.0104
Co	0.00365	0.0347	0.0152	0.0996	0.00384	0.0258	0.00928	0.0239	0.00258	0.0217	0.00781	0.0157
Ni	0.00658	0.0424	0.0205	0.0878	0.00516	0.0389	0.0191	0.00713	0.00153	0.0449	0.00885	0.00368
Cu	0.0896	0.679	0.260	0.166	0.0411	0.380	0.102	0.00209	0.000936	0.552	0.0107	0.00381
Zn	0.103	0.872	0.257	0.225	0.0553	0.409	0.131	0.0991	0.0505	0.440	0.150	0.186
Cd	0.0163	0.215	0.0731	0.0758	0.00486	0.293	0.0433	0.0588	0.00582	0.119	0.0363	0.0757
As	0.000316	0.0139	0.00559	0	0.00169	0.00956	0.00467	0.00432	0.00109	0.0207	0.00462	0.00582
Ag	0.00391	0.0923	0.0459	0	0.00426	0.0888	0.0308	0	3.95	0.0648	0.00611	0
Pb	0.00773	0.156	0.0711	0.0226	0.00118	0.0697	0.0113	0.00173	4.99	0.0256	0.00211	0.000581
Mo	0.187	2.14	0.515	0.658	0.116	1.17	0.270	0.435	0.104	2.50	0.592	3.25

Most of the minor elements had BFs up to 1 at all the three sites. The elements Sr, U, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn and Cd had very low BF values. The elements silver and lead had high BF values at site M that ranged up to 3.95 for silver and 4.99 for lead, why these elements are highly accumulated in pine needles at this site. Bioconcentration factor values for molybdenum at site L ranged from 0.116 (rather far from 1.0) to 1.17 which is close to 1 and is not so high. Bioconcentration factors had higher values at the other sites that ranged up to 2.14 at site H and 2.50 at site M. The following figures show the data for selected elements.

Fig. 5. Bio-concentration factor (BF) for trace elements Mo at sites H,L and M.



To summarize the results of minor/trace elements, molybdenum had high BF values at the three sites why this element is accumulated at all investigated sites. However, V, Pb and Ag had their high BF values only at site M.

The following tables 4,5,6,7,8,9 present the range of major and minor/trace elements content in this study at all sites:

Table 4: Range, standard deviation and mean of major elements contents in collected samples, site H (mg/kg)

Element	Major elements soil µg/g	Major elements needles µg/g	Standard deviation major elements soil µg/g	Standard deviation major elements needles µg/g	Mean major elements soil µg/g	Mean major elements needles µg/g
Ca	30022 - 79905	3112 – 14632	11699	2930	62139	7966
K	19.15 – 150	3698 – 7227	38.9	822	67.4	5323
Mg	9186 – 22398	1185 – 3160	3061	508	17094	2309
Na	89.61 - 351	53.46 – 92.19	89.6	10.3	197	72.2

Table 5: Range, standard deviation and mean of major elements contents in collected samples, site L (mg/kg)

Element	Major elements soil µg/g	Major elements needles µg/g	Standard deviation major elements soil µg/g	Standard deviation major elements needles µg/g	Mean major elements soil µg/g	Mean major elements needles µg/g
Ca	27194 - 62448	1819 - 11310	15597	2635	40029	6086
K	26.26 - 101	3564 - 8108	27.4	1521	51.5	5298
Mg	7447 - 18932	902 - 2571	471	436	11683	1753
Na	27.10 - 190	45.83 – 96.67	63.5	16.7	99.3	73.08

Table 6: Range, standard deviation and mean of major elements contents in collected samples, site M (mg/kg)

Element	Major elements soil µg/g	Major elements needles µg/g	Standard deviation major elements soil µg/g	Standard deviation major elements needles µg/g	Mean major elements soil µg/g	Mean major elements needles µg/g
Ca	35744 - 74404	1523 – 8908	15335	1577	47557	6005
K	4.34 – 30.75	3264 – 6073	11.4	797	17.2	4798
Mg	10324 – 15336	909 - 2542	2073	471	11887	1411
Na	4.68 - 153	59.82 – 104	55.03	13.2	63.1	80.7

Element	Range in soil µg/g	Range in needles µg/g	Standard deviation minor elements soil µg/g	Standard deviation minor elements needles µg/g	Mean minor elements soil µg/g	Mean minor elements needles µg/g
Sr	159 - 181	5.05 – 44.3	19.9	10.9	159	21.9
U	1.42 – 1.68	0.00168 –0.0360	0.233	0.00916	1.53	0.0205
Al	20920 – 27023	61.1 – 490	1945	95.5	23391	236
V	45 – 55	0.0230 – 0.887	4.11	0,224	49.4	0,451
Cr	39 – 49	0.0424 – 2.83	3.99	0.713	43.6	1.12
Mn	602 – 717	12.1 – 68	65.5	22.7	677	47.2
Fe	18080 – 21054	30. 1 – 809	1132	199	19342	353
Co	8.45 – 9.90	0.0517 – 0.294	0.635	0.0632	8.81	0.134
Ni	34.3 – 30.6	0.226 – 1.18	2.56	0.225	30	0.613
Cu	23.4 – 27.8	3.21 – 15.9	18.6	3.08	25.7	6.70
Zn	83.6 – 159	16.4 – 63.0	54.2	12.2	140	36.1
Cd	0.31 – 0.520	0.00843 – 0.0662	0.177	0.0134	0.44	0.0319
As	26 (LOD)*	0.00823 – 0.227	0,00	0.0875	26.00	0.145
Ag	0.26 (LOD)	0.000296 – 0.0232	0,00	0.00568	0.24	0.0126
Pb	18.2 – 20.4	0.158 – 2.84	29	0.673	19.4	1.39
Mo	0.585 – 0.769	0.144 - 1.23	0.0244	0.254	0.67	0.344

Table 7: Range, standard deviation and mean of minor/trace elements contents in collected samples, site H (mg/kg).

*for the elements not detected in soil the respective LOD value was used in the calculations.

Table 8: Range, standard deviation and mean of minor/trace elements contents in collected samples, site L (mg/kg).

Element	Range in soil µg/g	Range in needles µg/g	Standard deviation minor elements soil µg/g	Standard deviation minor elements needles µg/g	Mean minor elements soil µg/g	Mean minor elements needles µg/g
Sr	93.0 – 174	2.63 -19.3	26.3	4.95	119	9.91
U	0.930 – 1.50	0.000843 – 0.0273	0.194	0.00852	1.16	0.00991
Al	19791 – 24601	38.1- 380	2205	101	22334	137
V	47.2 – 62.6	0.00939 - 0.921	5.04	0.284	53.8	0.291
Cr	37.9 – 49.7	0.0455 – 1.96	12.5	0.628	44.3	0.756
Mn	638 - 869	10.3 – 77.7	85.9	19.9	755	31.9
Fe	16143 – 20864	28.06 - 581	14528	177	19196	220
Co	7.09 – 9.78	0.0376 – 0.183	1.66	0.0397	8.65	0.0803
Ni	27.1 – 36.0	0.186 - 1.06	38.4	0.274	31.7	0.606
Cu	32.0 – 75.2	3.09 – 12.2	931	1.95	57.5	5.85
Zn	94.1 – 257	14.2 – 38.5	60.1	5.95	200	26.1
Cd	0.287 – 0.906	0.00440 – 0.0843	0.282	0.0166	0.58	0.0251
As	26 (LOD)*	0.0440 – 0.249	0,00	0.0621	26.00	0.121
Ag	0.26 (LOD)	0.000678 – 0.0226	0,00	0.00675	0.24	0.00938
Pb	37.6 – 98.0	0.116 – 2.62	446	0.774	76,25	0.881
Mo	0.72 – 1.61	0.186 – 0.843	0.333	0.155	1,45	0.365

*for the elements not detected in soil the respective LOD value was used in the calculations.

Table 9: Range, standard deviation and mean of minor/trace elements contents in collected samples, site M (mg/kg).

Element	Range in soil µg/g	Range in needles µg/g	Standard deviation minor elements soil µg/g	Standard deviation minor elements needles µg/g	Mean minor elements soil µg/g	Mean minor elements needles µg/g
Sr	86.9 – 115	3.47 – 19.1	11.5	3.95	107	13.1
U	1.13 – 1.41	0.00143 – 0.0378	0.118	0.00821	1.28	0.0132
Al	16047 - 21871	51.55 – 193	2256	36.9	19700	115
V	44.1 – 54.8	0.00480 – 0.688	4.40	0.155	50.7	0.276
Cr	38.5 – 84.4	0.0750 – 4.52	19.3	1.08	50.1	1.13
Mn	550 – 740	13.39 – 92.34	74.00977	14.8	673	32.1
Fe	16530 - 69133	24.26 – 993	22566	234	28848	291
Co	8.28 – 13.7	0,0353 - 0,179	2.18	0.0341	10.1	0.0788
Ni	25.4 – 164	0.251 – 1.14	59.6	0.219	57.1	0.505
Cu	45.4 - 3277	3.07 – 25.1	1440	5.49	701	7.52
Zn	171 – 339	17.11 - 75.1	65.3	12.1	226	33.9
Cd	0.729 - 1.37	0.00800 - 0.0869	0.262	0.0197	0.92	0.0334
As	26 (LOD)*	0.0285 – 0.538	0,00	0.130	26.00	0.120
Ag	0.26 (LOD) – 5.08	0.000168 – 0.0157	0,00	0.00517	2.19	0.00750
Pb	64.1 – 158	0.0786 – 1.64	686	0.393	387	0.814
Mo	0.714 - 1.09	0.113 – 1.78	0.123	0.436	0.90	0.530

*for the elements not detected in soil the respective LOD value was used in the calculations.

As the data of this study do not include qualitative information about the metal speciation in the soil, the bioavailable fractions cannot be identified but can be estimated as shown below for selected elements. Zeiner et al (2013). reported in a previous study the chemical speciation of selected elements according to a modified BCR protocol [34]. The BCR protocol is an operational procedure used to identify principal metal species by extracting fractions in a series with different reagents. The acid soluble and exchangeable fraction was determined by adding 40 mL of CH_3COOH (0.11 mol L^{-1} per 1g of dry soil sample which was shaken for 16 hours at room temperature on an orbital shaker at 250 rpm [34]. The time trend for the extraction data was checked and no statistically significant time trends was found (as mentioned previously no statistically significant difference was found for samples in this study either). The results showed that using BCR fraction 1 the following elements amounts were extracted: K (27%), Sr (62%), Mn (32%) and Ni (6%). The most bioavailable fraction is the first fraction while the least bioavailable fraction is the last one [35]. An estimation of bioavailable fractionation for some elements at site M is presented in table 10 below:

Table 10: Bioconcentration factors for extracted fractions and total extraction yield using BCR

Element	Mean content in soil mg/kg	Extraction yield in BCR fraction 1 in % [34]	Extracted content in mg/kg	Mean content in needle in mg/kg	BF for fraction 1
K	17.3	27	4.67	4799	1027
Sr	107	62	66.3	13.1	0.19
Mn	673	32	215	32.1	0.15
Ni	57.1	6	3.43	0.51	0.15

The BF for potassium according to BCR fraction 1 at site M is 1027 (table 10), while the total BF for K is 278 (table 2). It can be noted that BF for a bioavailable fraction is much higher than that for total element. The total content of elements in soil helps in detecting the contamination intensity but using extracting agents to identify potentially toxic elements informs about main reactions that control the behavior of elements in soil [36]. Some metals do not affect ecosystems since they are strongly bound to the soil matrix, i.e. not accessible to organisms. However, these metals cause pollution when they are found in separate entities. [3].

Many studies have demonstrated the bioavailable fraction of elements in soils using different ways. Quevauviller [37] compared some of these different methods trying to find the best one for finding the bioavailable fraction of heavy metals in soils where he argued that EDTA (Ethylene Diamine Tetra Acetic Acid) extraction was a good method. In this study, the focus was on calculating bioconcentration factors for metal content in order to estimate the bioavailability. The results in this study showed that the bioavailable major metals ranged in the following order: K>Na >Mg>Ca. The bioavailable major metal fractions was high for potassium. It was difficult to find previous studies with similar results but there are many studies that estimated the bioavailable fractions of other metals. Elnazer et al [38] investigated soils contaminated with Pb, Cd, and Zn along Alexandria-Marsa Matruh highway, Egypt. The results of their study indicated bioavailable fractions of 72.5% for Pb and 37.5% for Cd contents. The results of Elnazer et al are different from those in this study and this could be related to the different areas investigated in each study. The mentioned metals did not indicate fraction bioavailability in this study.

Comparison with BF values reported in literature

The results in this study are compatible with the results of some studies such as a study done by Solgi et al. where they investigated some *Pinus eldarica* tree as bioindicator for Cu, Pb, and Zn in an urban area [38]. The calculations of BF values were done for samples of needles and of the bark of the pine tree. Solgi et al clarified that they calculated the values of BF in order to investigate the use of pine trees as bio-accumulators in the studied area.. The results of their study indicate that pine trees are accumulators for lead in the investigated area but not for zinc and copper. The BF values for copper and zinc in the needles were 0.44 and 0.6, where both are less than 1. The BF for lead was as high as 7.8 [39]. Alahabadi et al. investigated how heavy metals can be bio-accumulated by plant tissues from soil in the urban environment. Their study included fourteen tree species including *Pinus eldarica* [40]. This study indicated that the highest BF values of Zn, Cu, Pb and Cd for needles were in samples of *Pinus eldarica*. The BF for Zn, Cu, Pb, and Cd were in the range of 0.163–0.647, 0.005 to 0.281, 0.007 to 0.227, and 0.356 to 0.647 for needles. Alahabadi et al. concluded that *Pinus eldarica* trees are good accumulators for their investigated elements [40]. In another study, Sun et al. investigated the concentrations of sulphur and heavy metals in needles and soil of Masson pine *(Pinus massoniana L.)* trees in an urban-rural gradient [41]. The results showed that the Masson pine trees are good accumulators for zinc in the investigated areas. The values of the bioconcentration factors were in the order Pb < Cu < Cd < Ni < S, Cr, Zn, where the lowest values were 0.59-4.00 for lead [41].

Conclusion

This study investigated Pine trees as bioindicators in three areas with different traffic tact. BF were calculated to check the accumulation of every element in pine needles. Bioconcentration factors demonstrated that pine trees accumulated the major heavy metals K and Na at all the three sites. The lowest BF values for K and Na were in site H while the highest accumulation was in site M with very high values for K ranging up to 1400. Thus, pine trees can be considered as good bio-accumulator for K and Na where the accumulation ratio >was in the following order: M>L>H. Bioconcentration factors of trace elements also demonstrated that pine trees can be considered as good bioindicators for Mo at all sites where these metals were highly accumulated in pine needles. Pine trees are also bioindicators for Ag and Pb but only in site M. The bioconcentration factors for these heavy metals (Ag, Pb) were high only in site M. The accumulation ratio for these trace elements was in the following order: Site M: Pb>Ag>Mo. Mo in site L and H. The extent of metal pollution in different places is usually affected by heavy traffic and industrial activities where the highest metal concentrations are usually found in sites with industrial emissions _[42]. In this study, the highest accumulation factors were found in site M with medium traffic tact. However, it should be taken into consideration the possibility of having factories or industrial emissions in this site. The high accumulation of some metals in pine needles indicate the possibility of using pine trees as biomonitoring in similar places in urban areas where Pine trees can help decrease soil and atmospheric pollution resulting of heavy metals.

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Appendices

Needles data, ICPMS for the minor elements

Samplename	H11-1a … (all sample columns)
Li7(LR)	[illegible]
Be9(LR)	[illegible]
B10(LR)	[illegible]
Sr88(LR)	[illegible]
Ag107(LR)	[illegible]
Ag109(LR)	[illegible]
In115(LR)	[illegible]
Ba138(LR)	[illegible]
Pb208(LR)	[illegible]
U238(LR)	[illegible]
Al27(MR)	[illegible]
V51(MR)	[illegible]
Cr52(MR)	[illegible]
Mn55(MR)	[illegible]
Fe56(MR)	[illegible]
Co59(MR)	[illegible]
Ni60(MR)	[illegible]
Cu65(MR)	[illegible]
Zn66(MR)	[illegible]
Mo95(MR)	[illegible]
Mo98(MR)	[illegible]
Ag107(MR)	[illegible]
Ag109(MR)	[illegible]
Cd111(MR)	[illegible]
In115(MR)	[illegible]
Pb208(MR)	[illegible]
K39(HR)	[illegible]
As75(HR)	[illegible]
Se77(HR)	[illegible]
Se78(HR)	[illegible]
In115(HR)	[illegible]

Samplename	N1	N2	N3	N4	N5	N6	N7	N8	N10-1a	N10-2a+Na	N11-1a	N11-2a	N12-1a	N12-2a	N13-1a	N11-2a (rep	N13-2a	N17	Nb	Nc	Nd	Ne	Nf	Ng
Li7(LR)	0,751745	1,078808	0,627825	0,748896	1,077152	0,746916	0,63596	0,025754	<QL	0,634973	0,082983	0,724705	0,096609	0,48731	0,093194	0,685414	1,031897	0,680716	1,819679	1,149282	0,63097	1,089563	0,421474	1,579881
Be9(LR)	0,002365	<QL	0,001512	<QL	<QL	0,010705	0,010126	<QL	<QL	<QL	<QL	0,001587	<QL	<QL	<QL	<QL	<QL	<QL	<QL	0,012209	<QL	0,019717	0,00522	0,012887
B10(LR)	38,56686	46,43286	33,48061	25,26895	37,27119	39,01437	38,621	18,78941	14,21965	27,9801	24,71955	46,60926	31,01108	32,07194	25,9315	36,67419	39,05644	38,93164	36,12038	19,40408	17,80228	40,42003	27,53875	37,9838
Sr88(LR)	12,36333	12,52597	8,79824	13,47822	10,05039	13,01574	5,477316	3,848604	2,633204	13,5024	4,409229	7,16437	4,851173	4,294029	3,455311	6,772775	14,49334	15,26491	19,33714	13,3048	9,420601	14,14467	6,434349	18,78677
Ag107(LR)	0,005951	0,000942	0,003156	0,006419	0,008887	0,004908	0,009869	0,002678	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	0,005744	<QL	0,015301	0,010618	0,016359	0,027184	0,008396	0,014371
Ag109(LR)	0,002473	0,000646	0,002421	0,006339	0,005216	0,000149	0,007396	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	0,005007	<QL	0,012254	0,003427	0,01244	0,021988	0,00391	0,009913
In115(LR)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ba138(LR)	4,645276	4,086742	3,407551	6,571343	6,267856	3,580373	1,564129	0,981836	0,735017	4,084046	1,02797	2,215221	1,121042	1,628798	1,140281	2,554948	7,180947	5,290212	10,11586	10,58698	7,177672	6,169661	2,791728	10,41323
Pb208(LR)	0,574948	0,505935	0,499402	1,016481	1,232948	0,439675	0,30331	0,140266	0,122425	0,490984	0,129863	0,416912	0,123065	0,35986	0,315355	0,57165	1,411478	0,767144	2,246896	2,681829	2,239898	1,56187	0,812831	2,186445
U238(LR)	0,00748	0,005963	0,006915	0,012371	0,017656	0,004423	0,004044	0,000968	0,001009	0,004877	0,001164	0,00543	0,000843	0,004003	0,001958	0,006283	0,013538	0,00945	0,023337	0,025779	0,02338	0,020125	0,009481	0,027312
Al27(MR)	75,79784	61,98086	68,57246	147,8707	174,7972	<QL	38,63334	<QL	<QL	46,62282	<QL	42,55172	<QL	38,08413	<QL	59,62697	166,0764	82,93945	183,3038	275,6179	306,0666	195,6703	127,0509	379,6887
V51(MR)	0,194192	0,149641	0,189985	0,356601	0,469037	0,104697	0,096628	0,017332	0,024172	0,146855	0,024324	0,104061	0,009389	0,092701	0,051578	0,144151	0,448427	0,221012	0,546036	0,921451	0,886665	0,607441	0,338671	0,827586
Cr52(MR)	0,587522	0,452825	0,451862	1,116925	1,190965	0,327201	0,271292	0,055868	0,081581	0,389908	0,144744	0,375235	0,045537	0,320794	0,125214	0,426168	1,059575	1,310975	1,626704	1,959955	1,949084	1,504149	0,749244	1,620207
Mn55(MR)	45,47982	67,69587	24,46511	10,29431	13,31471	28,52884	23,7988	40,65848	22,55208	71,69619	30,51332	16,53059	35,0734	10,33684	17,38537	16,94504	30,19976	77,72163	10,65216	19,27337	32,13947	47,50994	16,52367	57,14797
Fe56(MR)	175,112	155,5038	153,43	326,8059	366,4022	106,6351	95,79604	36,87343	35,51408	130,6628	30,82207	120,9644	28,06067	109,8253	53,81856	135,8766	315,2762	179,7588	453,9046	566,4872	581,1624	350,3719	231,0109	549,4056
Co59(MR)	0,072264	0,057954	0,068166	0,076391	0,092037	0,045453	0,044901	0,054334	0,063645	0,063213	0,049575	0,043535	0,049042	0,03761	0,062686	0,048059	0,120677	0,078163	0,092527	0,166677	0,142325	0,113042	0,102101	0,183182
Ni60(MR)	0,455299	0,619712	0,290894	0,510388	0,571283	0,271601	0,58671	0,937095	0,634167	0,302326	0,787704	0,197728	1,055169	0,185839	1,018717	0,264954	0,633494	0,351048	0,630909	1,006955	0,866262	0,838401	0,700244	0,838193
Cu65(MR)	5,48171	5,361451	5,09876	6,000631	6,466199	4,506049	5,017373	6,747902	3,343213	4,319159	5,999189	3,991186	6,884151	3,092981	5,809767	4,094997	7,014529	4,682381	7,041432	12,15736	8,618092	7,189904	4,116819	7,459768
Zn66(MR)	29,83197	28,57381	24,43857	19,51931	22,4868	22,80128	23,96835	37,24129	22,63628	22,80502	28,89919	18,11673	29,8007	14,22246	29,66643	18,73365	26,17878	24,95681	30,55766	36,06954	27,90755	38,45891	24,60538	24,935
Mo95(MR)	0,296156	0,278292	0,246225	0,406424	0,401453	0,252611	0,3298	0,357036	0,258011	0,237289	0,292719	0,25258	0,357699	0,176188	0,273976	0,24381	0,438676	0,27111	0,435812	0,517028	0,559789	0,366917	0,866489	0,637279
Mo98(MR)	0,301704	0,264328	0,26531	0,381429	0,376821	0,22969	0,308531	0,372199	0,252422	0,265219	0,279054	0,236582	0,361904	0,19628	0,267117	0,228267	0,477095	0,267719	0,424794	0,526124	0,557744	0,356376	0,820495	0,68001
Ag107(MR)	0,003778	<QL	0,004113	0,006387	0,010779	0,002895	0,01338	0,002813	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	0,006159	<QL	0,019806	0,010661	0,010386	0,023156	0,003495	0,014075
Ag109(MR)	<QL	0,000446	0,001275	0,005604	0,008632	0,000902	0,006377	0,001914	<QL	0,002313	<QL	<QL	<QL	<QL	<QL	<QL	0,003021	<QL	0,014474	0,004329	0,011617	0,01827	0,00166	0,007562
Cd111(MR)	0,024272	0,018311	0,016248	0,028483	0,037216	0,018322	0,013815	0,017837	0,010668	0,023167	0,014896	0,009408	0,016043	0,004402	0,02121	0,010995	0,025128	0,025755	0,049609	0,084276	0,024585	0,033487	0,042097	0,032785
In115(MR)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pb208(MR)	0,540161	0,493108	0,480285	0,985058	1,218471	0,42916	0,292549	0,13742	0,109332	0,472993	0,119781	0,395444	0,120068	0,348456	0,302965	0,559279	1,365649	0,749869	2,087638	2,554139	2,110368	1,462132	0,771259	2,045496
K39(HR)	11183,57	10090,07	9303,145	8423,474	9119,688	10320,13	11480,62	14510,69	10426,93	7583,516	13644,41	13500,17	13197,61	10519,36	19661,53	11629,68	12556,57	8453,053	3546,921	4735,25	4520,134	5640,967	10104,73	6217,765
As75(HR)	0,122112	0,13588	0,073667	0,097932	0,12078	0,083674	0,091419	0,125761	0,044013	0,065712	0,073734	0,084861	0,056976	0,051254	0,077979	0,123075	0,177736	0,120742	0,226905	0,242779	0,157216	0,229399	0,081046	0,248624
Se77(HR)	0,162954	<QL	0,295309	0,041236	0,095198	0,019437	0,142277	<QL	0,025168	0,06496	<QL	0,096358	0,057211	0,079322	0,03827	0,051199	0,198412	0,225753	0,084553	0,232201	<QL	0,085298	<QL	0,2163
Se78(HR)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL
In115(HR)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Samplename	M1 … (all sample columns)
Li7(LR)	[illegible]
Be9(LR)	[illegible]
B10(LR)	[illegible]
Sr88(LR)	[illegible]
Ag107(LR)	[illegible]
Ag109(LR)	[illegible]
In115(LR)	[illegible]
Ba138(LR)	[illegible]
Pb208(LR)	[illegible]
U238(LR)	[illegible]
Al27(MR)	[illegible]
V51(MR)	[illegible]
Cr52(MR)	[illegible]
Mn55(MR)	[illegible]
Fe56(MR)	[illegible]
Co59(MR)	[illegible]
Ni60(MR)	[illegible]
Cu65(MR)	[illegible]
Zn66(MR)	[illegible]
Mo95(MR)	[illegible]
Mo98(MR)	[illegible]
Ag107(MR)	[illegible]
Ag109(MR)	[illegible]
Cd111(MR)	[illegible]
In115(MR)	[illegible]
Pb208(MR)	[illegible]
K39(HR)	[illegible]
As75(HR)	[illegible]
Se77(HR)	[illegible]
Se78(HR)	[illegible]
In115(HR)	[illegible]

Soil data, ICPMS for the minor elements

Samplename	H1 (repl)	H1	H5	H10	H13	H17	N1	N5	N10	H1 (repl)	N13	N17	M1	M5	M10	M13	M17	N13 (repl)
Sr88(LR)	158,50	149,47	157,01	155,61	155,16	181,37	135,05	109,70	174,47	159,32	104,08	93,03	114,95	112,55	86,90	110,83	110,74	97,58
Ag107(LR)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	9,95	<QL	<QL	<QL
Ag109(LR)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	9,88	<QL	<QL	<QL
Pb208(LR)	19,64	18,37	20,64	19,17	18,84	20,18	78,52	97,87	38,31	19,00	84,21	63,28	88,22	64,88	1614,20	84,31	81,28	95,34
U238(LR)	1,68	1,55	1,66	1,44	1,42	1,43	0,93	1,13	1,50	1,57	1,12	<QL	1,41	1,19	1,13	1,28	1,37	1,10
Na23(MR)	655,44	517,24	830,21	518,85	636,37	507,81	698,51	491,93	510,07	576,69	470,17	388,60	2301,45	649,49	659,49	679,76	697,12	546,56
Mg24(MR)	19445,39	19243,12	18825,93	19216,72	18735,52	26426,47	8189,14	9282,43	23438,51	19294,64	9694,91	7782,86	13699,25	18975,05	9692,36	11694,25	11155,67	8930,38
Al27(MR)	24123,22	21273,14	27022,52	20919,77	23893,59	24894,86	21311,24	23417,71	22688,13	21612,67	22197,08	19791,20	21870,90	16046,58	19949,40	19457,75	21176,29	24600,79
Ca43(MR)	81586,78	79547,23	78883,69	80855,02	77416,79	110917,68	46475,05	38226,34	89344,56	82095,10	38028,53	32590,74	55462,36	118195,80	37837,19	49725,96	52092,47	35550,60
Ca44(MR)	77939,42	75257,69	74076,03	76702,81	74046,94	105429,12	46073,23	37643,31	84302,49	78061,43	37357,19	31701,40	54291,67	112550,11	36542,91	49478,35	51437,86	34731,00
V51(MR)	52,25	44,65	54,95	44,94	50,40	51,86	51,19	57,31	51,49	46,72	52,78	47,18	54,83	44,12	48,82	51,10	54,38	62,60
Cr52(MR)	46,59	39,00	47,53	39,13	43,94	49,06	42,81	49,67	43,48	40,02	43,25	37,85	43,46	84,42	38,49	41,26	42,83	48,44
Mn55(MR)	716,80	696,40	693,42	697,87	619,98	601,94	777,78	818,53	656,56	715,21	766,65	638,30	739,93	721,41	550,41	671,96	680,60	869,20
Fe56(MR)	19761,71	18756,82	18709,48	18079,83	19099,77	21053,60	17800,25	20729,48	20563,44	19934,76	19073,83	16142,63	20591,46	16530,41	69132,59	18897,09	19089,98	20863,63
Co59(MR)	8,93	8,48	8,76	8,52	8,45	9,90	7,85	9,25	9,78	8,60	8,65	7,09	8,78	13,68	10,60	8,28	9,14	9,31
Ni60(MR)	29,50	27,91	30,61	29,38	29,29	34,32	29,95	36,01	31,49	28,80	32,19	27,12	32,51	163,64	25,38	31,29	32,69	33,64
Cu65(MR)	25,14	23,35	27,75	26,10	27,49	26,25	64,31	75,17	32,01	24,02	59,32	48,77	61,21	45,41	3277,45	64,18	58,34	65,57
Zn66(MR)	159,10	154,33	150,59	157,47	122,22	83,60	233,47	257,13	94,06	155,81	215,45	167,08	218,74	170,61	338,82	201,27	201,97	230,70
Mo95(MR)	<QL	<QL	<QL	<QL	<QL	<QL	1,58	1,54	<QL	<QL	1,41	1,40	0,84	0,68	1,02	0,83	0,89	1,31
Mo98(MR)	0,71	0,58	0,77	0,65	0,69	0,67	1,64	1,64	0,72	0,64	1,41	1,27	0,92	0,75	1,15	0,90	0,98	1,51
Ag107(MR)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	9,55	<QL	<QL	<QL
Ag109(MR)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	9,55	<QL	<QL	<QL
Cd111(MR)	0,51	0,42	0,46	0,52	0,31	0,37	0,61	0,91	0,29	0,48	0,63	0,42	0,89	0,76	1,37	0,73	0,85	0,63
Pb208(MR)	19,26	18,00	20,13	19,03	18,40	19,53	78,76	98,07	36,81	18,52	83,66	63,96	89,97	63,40	1535,33	84,12	78,81	92,31
K39(HR)	8760,02	7534,38	10199,23	8441,86	8976,35	8699,31	8227,98	8994,80	8228,52	7879,59	8395,08	8577,07	6791,02	5542,08	7401,46	6946,36	7703,43	9466,37
As75(HR)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	75,83	<QL	<QL	<QL

Data for the major elements needles

Sample ID	all data are mass fractions in mg/kg	Ca 317.933 r	Ca 422.673 r	K 766.491 r	Mg 285.213 r	Na 589.592 r
M1		4890,93205	4745,25196	5787,4529	1202,675591	103,6790622
M2		6765,82406	6593,53966	5342,7306	1064,599605	96,27672067
M3		6292,08042	6141,91414	4568,2444	908,9047069	82,19384538
M3 (repl)		5469,21746	5307,28842	5897,2041	980,6675643	101,4533966
M4		4837,25716	4696,4316	4793,1967	993,8258879	85,230355
M5		7912,98814	7713,49853	4909,5931	1326,506618	88,93164616
M6		4903,45921	4741,53854	5293,5484	1182,364278	96,82927279
M7		5110,84276	4945,21938	5373,21	1042,910236	96,27518503
M8		4479,91541	4341,52284	4938,7069	1020,804101	88,54972559
M10		9026,9793	8788,19437	5440,4473	2107,246427	96,76963802
M11		5399,47181	5236,51363	5357,4483	1397,439187	94,95180428
M12		4314,56181	4150,56189	5654,3985	1135,527335	75,52546647
M13		5879,91596	5681,55526	5634,8441	1193,962405	71,59035984
M17		6319,62913	6143,89891	4628,5805	1368,579951	59,81969992
Mgr11-1a		2766,96187	2662,08703	7027,4093	1415,942509	78,52110959
Mgr11-2a		7222,04613	7011,69365	4916,8242	1932,950259	63,74668839
Mgr12-1a		3261,21257	3133,11312	6579,664	2107,437073	87,68469994
Mgr12-2a		7854,53874	7638,64465	6072,6226	2177,284277	79,80779007
Mgr12-2a (repl)		7832,12145	7613,93841	5070,009	2426,309804	65,52003975
Mgr13-1a		3284,15358	3158,00357	5946,0279	1519,945504	73,12350585
Mgr13-2a		7622,50036	7408,85095	5075,8732	1843,357548	64,55936929
Ma		3654,92265	3674,78373	4048,9331	1223,786068	80,01287
Mb		5421,76826	5500,48947	3414,3236	2542,418761	66,01675832
Mc		6048,6603	6077,4308	3769,0564	1103,773359	72,4594963
Md		7272,6365	7278,70863	4220,3617	1195,485223	81,10494493
Me=Mgr		1533,76634	1512,46098	3318,1452	971,2398422	64,8185564
Mf		6609,30105	6569,22638	4506,0481	1488,771447	85,00447835
Mg		7100,38836	7062,95266	4069,6304	981,3620323	76,5490048
Mh		7233,03622	7209,47171	3263,7117	1691,834824	63,90791225
Mh (repl)		6783,20548	6716,78329	4177,6239	1598,162947	78,47796252
Mi		6711,85505	6632,61957	4349,4526	1692,649249	80,64589925
N1		6541,19875	6412,66744	3842,4072	1992,802445	64,50638342
N2		7034,97985	6872,59353	4841,142	2252,170077	75,05092107
N3		4840,27415	4705,07288	4782,7812	1475,369196	75,42545916
N4		7603,8556	7416,58927	4681,3355	1394,343684	70,34595112
N5		5171,99566	5050,65601	3747,5095	1238,36692	51,43219358
N6		6108,42862	5941,59215	4953,7239	1893,170303	69,7346603
N7		2824,0993	2739,67596	4004,1156	1454,117575	57,13088813
N8		1849,24883	1788,32975	4375,7715	1534,195989	63,12385317
N10-1a		1159,88016	1116,57424	3877,7993	1094,137807	52,69884933
N10-2a=Na		7071,97101	6920,10113	4168,4592	2110,678766	58,18801816
N11-1a		2044,94526	1991,25304	3470,2256	1440,489284	43,76017264
N11-1a		2033,61783	1970,4134	3781,4696	1439,852779	48,13260474
N11-2a		4134,12584	4019,2975	4081,5741	1286,83913	51,97104789
N11-2a (repl)		3968,07265	3851,86919	4261,4568	1246,504263	53,89081266
N12-1a		2093,07352	2035,74356	3974,8796	1761,895984	53,96490925
N12-2a		2382,54326	2310,14805	3564,3717	901,8100528	45,82874249
N13-1a		1586,90335	1544,94018	2924,8416	1245,444088	37,85754697
N13-2a		7259,28315	7127,06227	5084,0112	1942,50731	92,96280497
N17		8756,68761	8579,96591	4958,6891	2243,931219	93,70798932
Nb		10750,4905	10098,6725	7468,0263	1789,24948	90,50806536
Nc		9452,76888	8853,89766	8108,209	1818,364806	96,66877692
Nd		4307,14538	4028,68081	6689,3683	1756,081223	83,58864023
Ne		8043,66605	7570,37506	7385,9299	2571,026464	88,08828033
Nf		4352,08811	4071,48639	6970,5815	1785,058733	85,08184668
Ng		11657,91	10961,3545	7985,4474	2380,306163	94,39712461
H1(-2a)		9542,73307	9097,97602	4533,7455	2396,100487	55,38526088
H2(-2a)		9541,68138	9087,65143	5385,6997	2639,970613	65,26436074
H3(-2a)		11004,3209	10428,3574	5848,6384	2878,355116	67,2031516
H4(-2a)		6096,60319	5779,09742	4583,7745	1891,805942	53,45511794
H5(-2a)		5715,52602	5387,13167	5514,2369	2037,953408	65,17251038
H6(-2a)		9255,79612	8814,21255	4815,2303	2483,48322	56,69413832
H7(-2a)		10488,0686	9938,8843	5766,0516	2927,819304	66,51791747
H8(-2a)		10291,5687	9760,59424	5422,9595	2714,937355	64,26527793
H9(-2a)		8398,47982	7923,29015	5431,1905	1880,175	61,56887826
H10-2a = Hc		4790,65977	4656,77537	4636,5082	1970,316831	74,98831976
H11-1a		1606,22656	1511,38434	4110,9072	1069,541515	52,54594914
H11-2a		8682,04497	8513,67	4474,7577	2469,475846	75,94031983
H12-1a		1904,81711	1786,77934	4334,1334	1162,952691	55,50582382
H12-2a		14735,0175	14528,4791	4562,0064	3027,756597	80,20632818
H13-1a		3209,55062	3016,37328	5624,1678	1864,163877	66,55428777
H13-2a		10420,0766	10214,7968	4719,4628	2780,658833	80,1528388
H14(-2a)		14439,0386	14260,1708	5236,3548	3159,989232	86,48571555
H15(-2a)		6399,51449	6222,37357	5671,4725	2013,857776	92,19032328
H16(-2a)		5832,88806	5686,02015	4932,4557	2040,371884	79,84142498
H17(-2a)		10775,5886	10622,991	4398,4278	2918,011196	72,56757085
H18(-2a)		3158,82826	3064,42809	4212,5183	1405,691204	69,10972356
Ha		6899,52768	6682,65722	5363,1445	2444,234854	65,00683176
Hb		6756,88851	6541,80743	5777,4239	2033,317328	70,0433205
Hd		5197,43323	5007,75416	5777,747	1873,917036	71,30612307
He		5779,13754	5407,58774	7226,7391	2883,244167	88,12659122
Hf		5273,60027	4948,06634	6289,8341	1834,792178	81,26137705
Hg		10154,7997	9583,02995	6825,9047	2605,356523	87,85700509
Hg (repl)		10089,7839	9523,9348	6099,4511	2038,916843	78,72794895
Hh		4116,81084	3847,01728	5900,7705	1184,891355	76,21467448
Hi		7649,13872	7845,10177	3698,3506	2272,5331	71,77873836

Data for the major elements soils

Sample ID	all data are mass fractions in mg/kg	Ca 317.933 r	Ca 422.673 r	K 766.491 r	Mg 285.213 r	Na 589.592 r
H1		80952,2773	78857,0353	96,144952	22397,94772	323,0991537
H1 (repl)		30118,0225	29925,0436	70,368906	9185,872174	111,8352099
H1		80215,0986	77751,5696	83,909046	22037,53289	304,9526832
H1 (repl)		58361,5522	57281,3448	35,348415	15869,58144	93,95749751
H1		60427,1518	58194,3742	33,476891	16077,12347	128,9458281
H5		59399,0751	56876,4572	40,633715	15825,16988	143,7090457
H5		60114,4323	58242,8179	94,436393	16373,96707	234,9241823
H5		59705,6468	57246,6439	83,250633	16191,22533	193,2477823
H10		68926,9917	66734,827	77,950189	17724,62375	238,0616026
H10		67410,7342	64713,5403	70,207301	17250,20887	196,8019226
H10		61095,3501	59240,1582	20,838945	16230,6015	99,81032114
H13		57621,6074	55775,982	22,539534	15647,69735	89,61253633
H13		59065,4253	55527,6724	149,50746	16293,81808	351,1684996
H13		60392,3206	56392,1625	131,1982	16598,87835	316,4416688
H17		74301,4774	73156,9753	19,151384	20081,2273	124,0137598
H17		73357,0818	71060,514	49,102897	19726,16449	204,3835115
N1		29853,0173	29462,7675	101,33907	8711,754463	190,4189682
N1		30006,3742	29613,3073	85,623514	8751,679317	127,334796
N5		30033,6779	30089,7149	51,8431	9181,058293	57,87031844
N5		58205,3518	56878,3565	68,045226	15750,79926	187,2966098
N10		63196,0479	61700,4968	37,817596	18932,19606	129,928903
N10		62698,7171	61482,298	28,150294	18790,87089	97,54136719
N13		31380,9649	31466,8587	26,259537	8945,01521	30,95545763
N13(repl)		30132,721	29942,5919	32,515656	8635,318792	45,60050325
N17		27235,1598	27153,238	32,066753	7446,850358	27,09682025
M1		44181,5613	43715,6461	8,5699988	12260,8229	41,07732798
M5		73805,4409	75003,1918	15,839754	15335,86986	153,3827619
M10		35287,482	36200,8111	4,3434158	10324,07795	4,678699141
M13		41046,4112	40874,2244	26,85556	11039,33919	55,15625819
M17		42988,3002	42471,7146	30,750481	10474,21324	61,40210214