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Bromine Accumulation in Some Crops and Grasses as Determined by Neutron Activation Analysis

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ABSTRACT

Till now information on bromine (Br) in the environment is still incomplete. The purposes of this research were the following: to study accumulation of Br in plants grown under different environmental conditions and to assess the factors controlling Br uptake by different plant species when the plants grow in soil uncontaminated with Br. For the determination of Br and other elements, neutron activation analysis was used. This method allows for determining a wide range of elements in various samples with high sensitivity and accuracy. Model tests and greenhouse experiments demonstrated different abilities of plants to uptake Br from various media and transfer it from roots to upper plant parts. It was shown that the main source of Br for a plant was soil. As a result of the plant growth concentration of Br in the rhizosphere soil decreased. The characteristics of soil have a pronounced effect on the Br uptake by plants.

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Bromine; neutron activation analysis; plant uptake; soil characteristics

Introduction

Although by now a lot of experimental studies on biogeochemistry of halogens and their influence on the environment have been carried out, available information is still uncompleted. A great deal of data about chlorine has been collected. However, it should be remembered that despite similar chemical properties of halogen elements, each of them has characteristic effects on plants and soils (Yuita et al. 1982).

Among the recognized trace elements, bromine (Br) is one of the most abounded and widespread in the biosphere. Although many plant species can accumulate large amounts of this trace element (sometimes concentration of Br in plants may be higher than its concentration in soil where the plants grow), the role of Br in various biochemical processes is still unknown.

High concentrations of Br in soil are typical for coastal areas. An increase of Br in the soils may be due to Br evaporation from the sea. Yuita (1983) reported that soils near the Sea of Japan accumulated up to 495 mg kg^{-1} Br. Cortizas and co-authors (2016) studied Holocene soils from an oceanic area of Western Europe. Bromine concentrations in the silt + clay (<50 μm) fraction in the soils were up to 778 mg kg^{-1}. Volcanic ash soils also contain rather large amounts of Br because of elevated Br contents in parent material and impact of volcanic exhalation (Kabata-Pendias 2011). Yamada (1968) reported that content of Br in humic volcanic ash soil sometimes can reach values as high as 850 mg kg^{-1}. An additional factor affecting an increase of the soil Br content is soil fumigation (Puri 2016). Considerable amounts of Br in agriculture soils may be provided by application of pesticides (fungicides, herbicides, and insecticides). Another source of Br in the environment is a wide use of brominated flame retardants (Hale et al. 2006).

The reported concentrations of Br in terrestrial plants vary from <1 to 285 mg kg^{-1} (Gan et al. 1998; Kabata-Pendias and Szteke 2015; Låg and Steinnes 1977; Sahin et al. 2012; Pourimani et al.

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2013; Shtangeeva et al. 2017; Tensho 1970; Wishkerman 2006). Contamination of soils with Br stimulated experimental studies and resulted in many publications on accumulation of Br in plants growing in coastal areas and in soil contaminated with different organic and inorganic Br compounds. A large part of the research was performed by Japanese scientists. However, less information is available on distribution of Br in different plant species growing in soil uncontaminated with Br. Besides, factors affecting uptake of Br by plants are not clearly understood. Probably, this may (at least partly) be due to rather low Br concentrations in such soils and plants. As a consequence, certain problems can arise at the stage of elemental analysis. There are also some problems associated with sampling, storage, and especially preparation of plants and soils for analysis. Various approaches used at these steps sometimes prevent comparison of the data obtained by different researchers.

Till the present time an accurate and sensitive measurement of Br in plant and soil samples represents certain challenges. Now, there are several techniques that may be used for Br determination.

Inductively coupled plasma–mass spectrometry (ICP-MS) was widely used to determine trace levels of halogens in plant and soil material (Barbosa et al. 2013; Bu, Wang, and Hall 2003; Mesko et al. 2016). However, main problem of this method is the necessity to transfer solid samples, including soils and plants, to liquid state. During this step there may be loss of volatile elements such as Br and other halogens. Besides, the digestion of soil samples not always results in transfer of all soil material to solution. Sometimes, if non-extractable forms of Br are present in the soil, it is difficult to determine their total concentrations in the samples.

X-ray fluorescence (XRF) spectrometry allows for rapid and direct measuring Br. However, sometimes sensitivity and accuracy of the XRF may be unsatisfactory for determination of low concentrations of Br in plants and soils. Besides, this technique is very sensitive to matrix effects, especially in the case when concentrations of elements are close to the limit of detection (Taurino et al. 2014). Certain errors can also occur because of the relationship between the thickness of the specimen and the Br concentration determined by the XRF (Gorewoda et al. 2015).

Neutron activation analysis (NAA) can provide a good opportunity to determine with high sensitivity and accuracy a wide range of elements (including Br) in various environmental samples. Besides (and it is very important), NAA may be performed directly. Thus, the analytical errors that might arise during preparation of samples for analysis may be significantly reduced.

For our experiments we used young seedlings because initial stage of the plant development is the most metal-sensitive (Bajji, Kinet, and Lutts 2002) and therefore, the most promising for such kind of research. Main crop species (wheat, barley, and oats) were used for the model vegetation experiments. In the course of field trials widely distributed weeds (couch grass and plantain) were collected.

The purpose of the research was to identify the conditions that can affect the uptake of Br by plants, compare the uptake and accumulation of Br by different plant species, and analyze the factors that control Br accumulation in different plant species when the plants grow in uncontaminated soil.

Materials and methods

Experimental design

Model tests

Seeds of wheat *Triticum vulgare (vill) Horst* were germinated for 6 days on a wet filter paper at room temperature. Uniform germinated seedlings were divided into 4 equal parts and transferred to pots filled with soil (2 kg in a pot) and jars filled with distilled water, water taken from a spring and nutrient solution of Hoagland (3 L in a jar). The modified Hoagland's solution had the following composition: potassium as KNO_3 at 334 μM, calcium as $Ca(NO_3)_2$ at 68 μM and magnesium as $MgSO_4 \cdot 7H_2O$ at 82 μM. The spring was far removed from possible sources of pollution. Soil was taken near the spring from a top (0–10 cm) soil horizon. The plants were grown in the soil and in the liquid media during 7 days.

Greenhouse experiments

Soil for the experiments was taken from upper (0–10 cm) horizon at 3 sites located along one street in St. Petersburg. Characteristics of the soil taken from the sites were different. The collected soils were placed into ceramic pots (5 kg in a pot). Seeds of oats *Avena Sativa* L. and barley *Hordeum vulgare* L. were germinated during 5 days and then grown in the pots during 19 days.

Field trials

Field experiments were carried out in St. Petersburg. Four small (1 m x 1.5 m) plots were used for the trials. Soil and plants (couch grass and plantain) were collected from the sites in the beginning of May 2015.

All of the experiments were performed at least in triplicate.

Elemental analysis

Plants were washed carefully just after sampling with ultrapure water to remove dust and small particles of soil from the plant surface. The soil and plant samples were air-dried at room temperature up to a constant weight. Instrumental neutron activation analysis was used to determine Br and other elements in the soil and plants. Samples were weighed, placed in quartz ampoules and irradiated in a nuclear reactor. Soil samples were irradiated for 18 h and plant samples for 24 h in a thermal neutron flux of 1×10^{14} n cm^{-2} s^{-1}. In the research, the samples were irradiated using three nuclear reactors: the DR3 heavy-water moderated reactor of Risoe National Laboratory, Denmark (Shtangeeva et al. 2001); the CEA/Saclay nuclear reactor OSIRIS, France (Shtangeeva and Ayrault 2004); the Research Reactor BER II of the Hahn Meitner Institute Berlin GMBH, Germany (Shtangeeva et al. 2009). Spectra of the irradiated samples were measured using a germanium detector (volume 178 cm3, efficiency: 35%, FWHM: 1.79 keV at 1.3 MeV). For determination of Br was used isotope ^{82}Br. The limit of Br detection was 0.1 mg kg^{-1} for plants and 0.3 mg kg^{-1} for soils. These levels were lower than ordinary Br concentrations in the plant and soil samples. The certified reference materials (CRMs) NIST 1573 (tomato leaves) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and SOIL-7 (International Atomic Energy Agency) were analyzed together with plant and soil samples. The results of the analysis of the CRMs showed a good agreement with certified values (Table 1). Concentration of Br in liquid media was determined by ICP-MS. The ICP-MS analysis was performed using a Termo Finnigan model Element 2 instrument (Bremen, Germany). The instrument was equipped with a concentric PFA-ST nebulizer connected to a Scott PFA spray chamber, platinum sample and skimmer cones and a demountable torch of quartz with guard electrode. The instrument was calibrated using 0.6 M HNO_3 solutions of multi-element standards. An aqueous certified reference material (SPS-SW-2, Spectrapure Standards, Norway) was analyzed at the beginning and at the end of each analytical sequence to check the calibration of the instrument.

Data analysis

For multivariate statistical analysis, Statistica for Windows 6.0 Software packages (StatSoft, Tulsa, OK, USA) were used. Calculation of the mean concentrations of elements and the analysis of variances to estimate the differences between the groups of samples were carried out. Additionally,

Table 1. Results of Br determination in CRMs Tomato leaves 1573 and Soil-7.

	Number of samples	Unit	Measured	Certified
Tomato leaves	10	mg kg^{-1}	27 ± 4	26 ± 3
Soil-7	6	mg kg^{-1}	6.8 ± 0.8	7 ± 1

Pearson correlation analysis was applied to the experimental data sets to assess a contribution of the specific factors that may have an effect on the uptake, distribution and relationships between Br and other elements in different parts of the plants and in soil, thus providing a better insight into the processes of element uptake and the relationships between elements typical for each plant species.

Results and discussion

Uptake of Br by the plants grown in soil and in liquid media

Model tests with wheat seedlings revealed different ability of the plants to uptake Br from various media and transfer it from roots to upper plant parts (Figure 1). Compared to the plants grown in liquid media, Br concentration in leaves of the soil-grown plants was the least (differences between Br concentrations in leaves of the plants grown in soil and in different liquid media were statistically significant at $P < 0.01$). The differences between the concentration of Br in the roots and in leaves of wheat seedlings were not significant with exception of the experiment with plants grown in nutrient solution of Hoagland. In this case, mean Br root concentration (6.5 mg kg^{-1}) was statistically significantly ($P < 0.05$) lower than mean concentration of Br in leaves (10.1 mg kg^{-1}).

Initial (before planting) concentration of Br in wheat seeds was 3.52 mg kg^{-1}. In seeds of 13-day-old wheat seedlings the Br concentration was 4.01 mg kg^{-1} (differences between these concentrations were not statistically significant). Bromine content in the initial soil (before plants were transferred to the soil) was 4.03 mg kg^{-1}. As a result of the plant growth the concentration of Br in the rhizosphere soil of the wheat seedlings decreased statistically significantly ($P < 0.05$) to the level of 3.26 mg kg^{-1}. This may indicate that main part of Br was taken by plants from soil. The concentrations of Br in the liquid media were rather low: 7.4, 6, and 3.7 μg L^{-1} in nutrient solution of Hoagland, spring water and distilled water, respectively. Nevertheless, plants were also capable of accumulating rather high concentrations of Br. This was probably due to the presence of Br in the solutions in a form that was easily available to plants. One important point to remember is that experimental data on accumulation of different elements obtained for the plants grown hydroponically must be used with a caution to predict uptake of the macro- and trace elements by the plants grown in soil. As an example, Figure 2 illustrates concentrations of K in roots of the wheat seedlings grown simultaneously in the different media. Differences between K contents in the plants grown in soil and hydroponically were statistically significant at $P < 0.001$ (except roots of the plants grown in spring water which was taken from the same place as soil). Similar behavior was also observed for other essential plant nutrients.

Effect of soil characteristics on the Br uptake by plants

The accumulation of Br in plants depends on various factors. Among the main factors, type of soil where the plants grow is one of the most important. Meanwhile, some authors (e.g., Wilkins 1978) stated that Br concentrations in plants do not correlate with concentrations of Br in soil and soil properties (pH, type, drainage status). Kabata-Pendias and Szteke (2015) suggested that concentration of Br in plants depends only on the concentration of the element in soil. Other researchers, however, reported the dependence of Br plant uptake on the soil parameters. For example, it was shown that uptake of Br was significantly smaller in the plants grown in poorly drained soils compared to the plants grown in well drained soils (Schnabel, Stout, and Shaffer 1995). It was also found that Br soil concentrations were positively correlated with clay content and negatively correlated with sand content of the soil (Gerzabek et al. 1999).

In order to assess whether there is any effect of soil parameters on the accumulation of Br in plants the following experiments were carried out. Two plant species – oats and barley – were grown in three different soils (some characteristics of the soils are present in Table 2). The soil pH values were 7.25 (site 1, loamy sand soil) and 7.6 (site 2, clay loam soil and site 3, sandy loam soil).

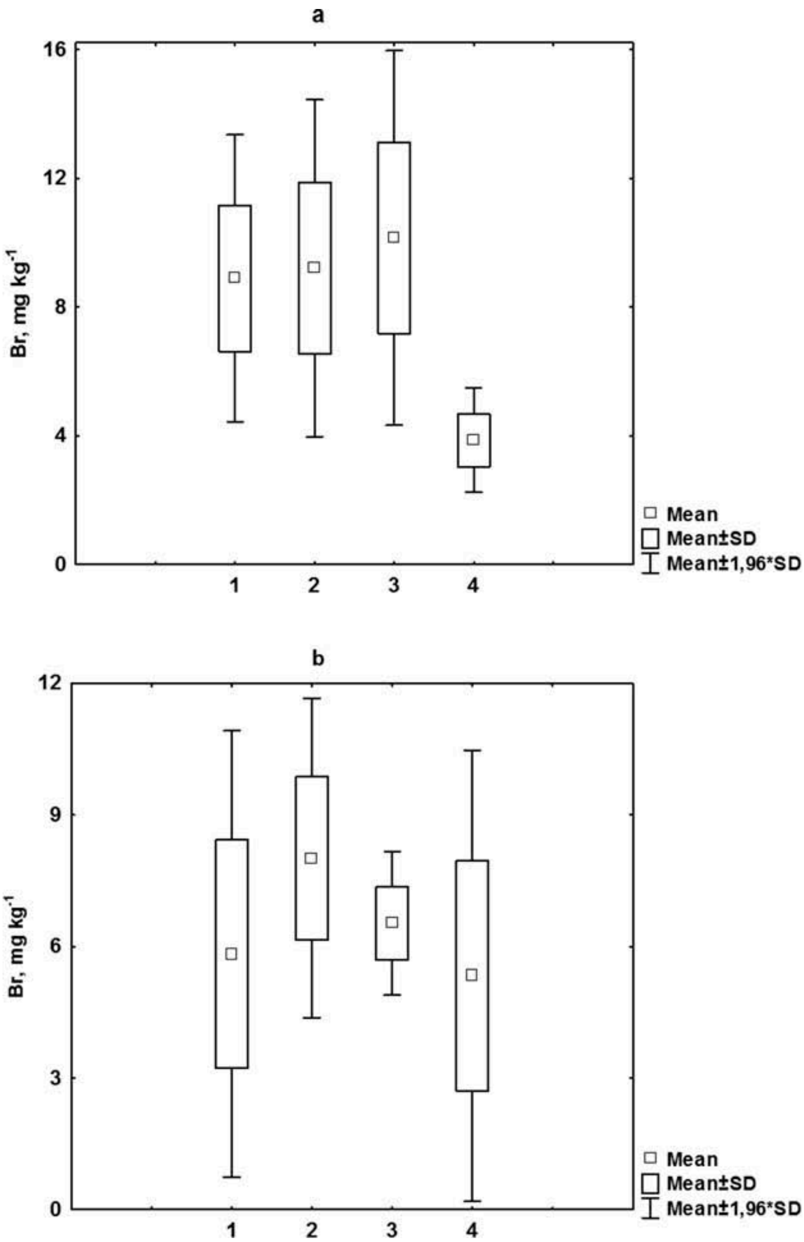

Figure 1. Bromine in leaves (a) and roots (b) of the wheat seedlings grown in distilled water (1), spring water (2), nutrient solution of Hoagland (3), and soil (4).

Concentrations of exchangeable cations (K, Ca, and Mg) in the soil taken from site 1 were much higher than in the soil taken from sites 2 and 3. The highest Br concentration was found in the loamy sand soil. The concentration of Br in the soil was higher ($P < 0.05$) as compared to Br content in the clay loam and sandy loam soils (Table 3). However, both oats and barley grown in the clay loam and sandy loam soils were capable of taking up more Br than the plants grown in the loamy sand soil where concentration of Br was higher. Moreover, concentration of Br was higher not only in roots but also in leaves of the plants. The differences between Br concentrations in the plants grown in the different soils were statistically significant at $P < 0.01$. Oats and barley grown in the loamy sand and

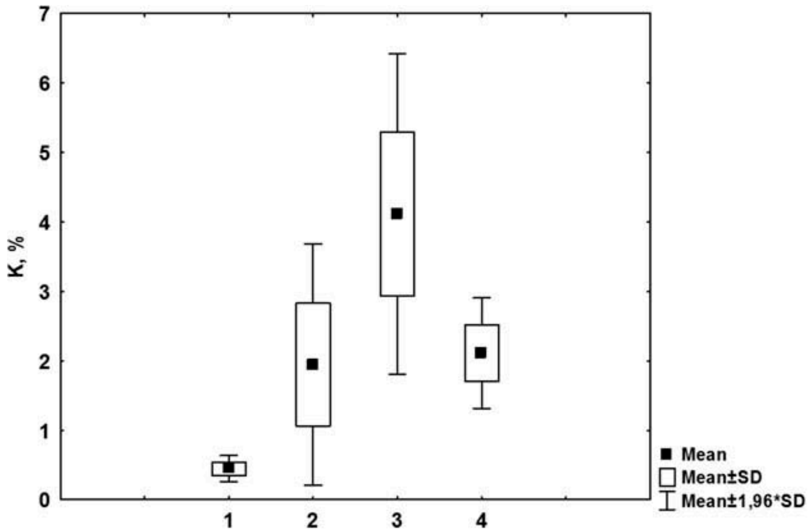

Figure 2. Concentration of K in roots of the wheat seedlings grown in distilled water (1), spring water (2), nutrient solution of Hoagland (3), and soil (4).

Table 2. Soil characteristics (greenhouse pot experiments).

	Site 1	Site 2	Site 3
pH (1:2.5 H_2O)	7.25	7.6	7.6
Texture	Loamy sand	Clay loam	Sandy loam
Exchangeable cations			
K^+, mg kg^{-1}	1160	340	800
Ca^{2+}, mg kg^{-1}	2510	1865	1925
Mg^{2+}, mg kg^{-1}	685	135	170

Table 3. Mean (of 7 replicates) concentrations (mg kg^{-1}) ± SD of Br in soil and in leaves and roots of oats and barley grown in the soil.

		Site 1	Site 2	Site 3
Oats	Rhizosphere soil	4.46 ± 0.97	2.12 ± 0.50	3.07 ± 0.88
	Roots	3.31 ± 0.73	8.28 ± 2.50	15.1 ± 1.4
	Leaves	2.31 ± 0.61	9.69 ± 2.28	24.8 ± 3.9
	Ratio of Br (roots/soil)	0.74	3.9	4.9
	Ratio of Br (leaves/roots)	0.70	1.2	1.6
Barley	Rhizosphere soil	4.47 ± 0.61	2.83 ± 0.59	2.90 ± 0.44
	Roots	2.66 ± 0.56	6.72 ± 3.16	22.0 ± 7.4
	Leaves	2.58 ± 1.33	9.75 ± 3.92	42.6 ± 6.1
	Ratio of Br (roots/soil)	0.59	2.4	7.6
	Ratio of Br (leaves/roots)	0.97	1.4	1.9

clay loam soils accumulated similar amounts of Br. The same plants grown in the sandy loam soil, however, differed in the ability to uptake Br: barley accumulated more Br than oats.

The ratios of Br concentration in the plant roots to its concentration in soil were the lowest for the plants grown in the loamy sand soil and the highest for the plants grown in the sandy loam soil. Similar trend was observed for the ratios of Br concentration in leaves to its concentration in roots (Table 3). Thus, it may be assumed that in the case when Br concentrations in soil are not rather high, the level of Br uptake by plants depends first of all on the soil parameters and sometimes no correlation between soil Br concentration and concentration of Br in the plants may be found.

Table 4. Mean concentrations of Br (mg kg^{-1}) ± SD in soil and in grasses (field trial). In parentheses are number of samples.

	Plantain		Couch grass	
Soil (40)	Roots (20)	Leaves (20)	Roots (20)	Leaves (20)
3.86 ± 1.02	2.73 ± 0.79	1.64 ± 0.35	4.36 ± 1.28	1.25 ± 0.76

Field experiments

For field trials, two widespread weeds (plantain and couch grass) were used. The plants were collected in urban area (St. Petersburg). The soil was podzolic with clay texture. Concentrations of Br in the plants are shown in Table 4. The experiments demonstrated certain differences between Br accumulation in natural grasses and cultured plants. The root Br concentrations in wheat, oats and barley were either similar or lower in comparison with the concentrations of Br in leaves (Figure 1 and Table 3). The concentrations of Br in the leaves of plantain and couch grass were statistically significantly (P < 0.01) lower than Br concentrations in the roots (Table 4).

Our experimental data demonstrated that sometimes concentration of Br in plants may be higher than in soil where the plants grow. It is hardly possible to suggest that the accumulation of Br in the leaves was due to its entry from the air since all the plants were collected far away from any source of Br to the atmosphere. It should be noted that accumulation of this trace element in the plants grown in uncontaminated soils occurred against concentration gradient. It seems that plants are capable of providing a specific amount of Br in their tissues. Probably, this may indicate that Br plays certain role in the biochemical processes in different plants, although now this may be only a suggestion. On the other hand, it is also possible to use the ability of plants to uptake Br from contaminated soils to clean up the soils. Several authors reported about significant decrease of soil Br concentration resulting from Br accumulation in plants (Masui et al. 1978; Shtangeeva et al. 2017).

Relationships between Br and other elements

Table 5 illustrates results of correlation analysis of element concentrations in plants (on the example of couch grass and plantain). There was no correlation between Br and other elements in roots of couch grass. In roots of plantain, only one statistically significant (P < 0.05) correlation between Br and sodium (Na) was observed. However, in leaves of the plants Br was highly correlated with many elements and these correlations were similar for leaves of both couch grass and plantain. In soil, the only statistically significant positive correlation - between Br and antimony (Sb) was found. Thus, it may be suggested that behavior of Br in soil, roots and leaves of the plants growing in the soil may be different.

Table 5. Statistically significant (P < 0.05) coefficients correlation between Br and other elements in couch grass and plantain.

	Couch grass		Plantain	
	Roots	Leaves	Roots	Leaves
Sodium		0.47	0.43	
Potassium		0.72		0.51
Calcium		0.68		0.44
Chromium		0.61		
Cobalt		0.63		
Rubidium				0.83
Strontium		0.56		0.55
Antimony		0.70		0.62
Cesium				0.64
Barium		0.75		0.79

Conclusions

Plants are capable of accumulating large amounts of Br. When plants grow far away from any source of Br, probably, the main contribution of Br to the plant supply may be expected from soil. Soil characteristics can significantly influence on the uptake of Br by plants. Under ordinary conditions (when plants grow in uncontaminated with Br soil) sometimes there is no correlation between soil Br concentration and concentration of Br in plants.

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