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Effects of bromides of potassium and ammonium on some crops

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ABSTRACT

In this work, the response of wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.), and pea (*Pisum sativum* L.) to bromides of potassium (KBr) and ammonium (NH₄Br) was studied. All plants were capable of accumulating high concentrations of bromine (Br). However, the Br accumulation depended on the Br compounds presented in the growth medium and plant species. The highest Br concentrations were observed in leaves and roots of the seedlings germinated in the medium spiked with KBr. Oat accumulated more Br than other plants and the lowest Br accumulation was observed in pea. The bioaccumulation of Br resulted in suppression of plant biomass and concentrations of several essential nutrients (K, Na, Ca, Mg, Zn and Cl). The most negative effects were caused by NH₄Br. Probably, this action was due to cumulative effects of Br⁻ and NH₄⁺. Among other plant species, the most tolerant to bromides was oat and the most sensitive was wheat.

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bioaccumulation; nutrients;
plant biomass

Introduction

Bromine (Br) is the 44th most common element in the Earth's crust. It occurs widely as soluble bromide salts in sea water, salt lakes, and underground brine deposits associated with oil. There are also numerous known organobromine compounds synthesized by living organisms or formed during natural abiotic processes (Moreno et al. 2015). Bromides are commonly found in the environment along with sodium chloride, owing to their similar physical and chemical properties, but in smaller quantities.

Worldwide production of Br compounds is estimated to be around 330,000 tons per year (Ross 2017). Meanwhile, it was shown that bromides are persistent in the environment, accumulate in the food chain and are of toxicological concern. The application of Br-containing chemicals in industry and agriculture resulted in an increase of Br content in soil (Honaganahalli and Seiber 1996; Venkatesan and Halden 2014; Wisniak 2002). Plants growing in the soil demonstrated a considerable accumulation of Br compared to concentration of the trace element in plants growing in clean soil (da Rosa Couto et al. 2018; Djingova et al. 1986; Eljarrat et al. 2008; Huang, Zhang and Christie 2011; Mueller et al. 2006; Yang et al. 2017).

It was shown (McCall et al. 2014) that Br is a new essential trace element for humans and animals. Although various plant species are capable of accumulating high concentrations of Br, according to our knowledge, its role in plants has not been identified. At the moment we can state only that Br is a constituent of all plant tissues. However, there is no real evidence yet to

support the view that Br is concerned with any function in a plant. Thus, the question why plants have evolved the accumulation mechanism for Br as well as Br requirements of different plant species and its possible toxicity should be considered.

The aims of the research were (1) to assess the response of several plant species to the increase of Br concentration in the growth medium and (2) to study the factors responsible for uptake and accumulation of Br in the plants that were exposed to bromides of potassium (KBr) or ammonium (NH_4Br). The purpose was to estimate effects of both Br^- and other components (cations) of the compounds on the plant development and nutrition. The challenge was to test if the cations influence on the distribution of Br in the plants.

Among selected bromides, potassium bromide was used more often for experiments on uptake of Br by plants (Berry and Steward 1934; Buwalda, Stribley and Tinker 1983; Schnabel, Stout and Shaffer 1995; Sharratt and Knight 2005). Less information is available on effects of ammonium bromide on plants (Jacoby 1965; Wetchagarun et al. 2017). It is known that NH_4Br fully dissociates in aquatic environment to bromide and ammonium ions (Speight 2016).

The young seedling stage is the most nutrient-sensitive stage for a plant (Lee et al. 2007; Shamshad et al. 2018). Therefore, it would be useful to assess possible effects of Br exposure in the course of the first stages of the plant development.

Materials and methods

Experimental design

Seeds of wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.), and pea (*Pisum sativum* L.) were surface sterilized by two 3-min soakings with gentle stirring in commercial bleach (5.25% NaOCl). The seeds were then rinsed three times in sterile double-distilled water to remove surface sterilization agents. After surface sterilization, the seeds were placed on a filter paper in Petri dishes (diameter 150 mm, height 30 mm). The filter paper was moistened with a sufficient amount of water or with water spiked with either KBr or NH_4Br . The chemicals were acquired from Roschimreactive, St. Petersburg (Russia) as pure standards (99% of purity). Concentration of Br in the growth media was 50 mg L^{-1} . After 5 days, first series of plant samples was collected, and the rest of the seedlings was transferred into plastic pots filled with 1 L of water without bromides or water spiked with the same amount of KBr or NH_4Br . The seedlings were grown in the pots during 8 additional days. We did not add any nutrients to the growth media in the course of the experiments. The temperature in a naturally illuminated greenhouse was typically 25°C during the day (16 h light period/day) and 22°C at night. At the end of experiments the pH (1:2.5 H_2O) of the growth media was determined. The experiments were performed in triplicate (three replicates in one experiment). The plants (leaves and especially roots) were washed carefully just after sampling and air-dried up to constant weight.

Elemental analysis

Concentrations of Br, chlorine (Cl), and iodine (I) in the plant samples were determined by ICP-MS and concentrations of sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), and zinc (Zn) were determined by ICP-OES after leaching the samples with tetramethyl ammonium hydroxide at mild temperature using the method described by Tagami et al. (2006). Detailed description of the procedure is given in our previous publications (Shtangeeva et al. 2015; Shtangeeva et al. 2017). Quality control was performed through concurrent analysis of certified reference material (CRM) NIST SRM 1573a (Tomato leaves). The results obtained for the CRM were in a good agreement with the reported certified and informative values.

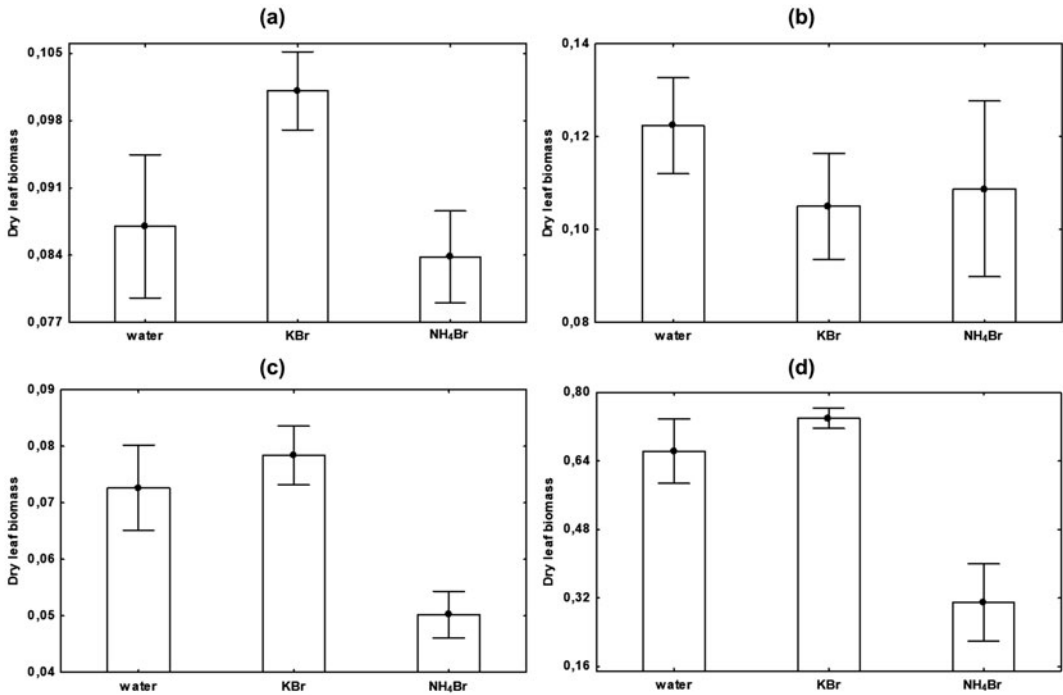


Figure 1. Dry biomass of leaves of rye (a), wheat (b), oat (c), and pea (d). The seedlings were germinated in water or in the media spiked with KBr and NH₄Br. The bars – mean dry biomass, T - mean \pm 0.95 confidence interval.

Data analysis

The experimental results were analyzed by variance analysis using the least-significant difference test (Statistica for Windows 6.0 Software packages, StatSoft, Tulsa, OK, USA). The differences between groups of samples were considered significant at $P \leq 0.05$ and the means were separated using a Fisher's protected least significant difference (LSD) test. Additionally, Pearson correlation analysis was applied to the data sets to assess the contribution of specific factors that may have an effect on distribution and relationships between Br and other elements in different parts of the plants. The correlation coefficient (r) and its significance was tested by t -test at 5% probability of error.

Results and discussion

Effects of Br on the biomass of plants

Physiological measurements of plants often are based on the plant growth or growth inhibition. According to available literature, the effects of Br on the plant development may be rather different and result in either suppression or stimulation of the plant growth (Baso-Cejas et al. 2007; Fransi et al. 1987; Pawłowska and Biczak 2016; Shtangeeva et al. 2015; Stelmach 1959).

Our previous experiments with wheat and pea seedlings grown in the water spiked with KBr demonstrated that this bromide did not affect negatively the plant biomass (Shtangeeva et al. 2015). As it is seen from Fig. 1, similar situation was observed for rye and oat seedlings germinated in the medium spiked with KBr. Leaf biomass of the rye seedlings germinated in the medium enriched with KBr even increased compared to biomass of leaves of the rye seedlings germinated in the medium not spiked with bromides. It was reported that the germination of 11 different crop species was not inhibited by KBr at concentrations below 500 mg L⁻¹ (Bowman

Table 1. Mean concentrations (n=3) of elements in plants (Br – mg kg⁻¹, other elements – g kg⁻¹). 1 – no treatments, 2 and 3 – the growth media were spiked with KBr and NH₄Br, respectively.

	Roots			Leaves		
	1	2	3	1	2	3
Oat						
Br	33 ± 6	26470 ± 450*	10020 ± 140*	37 ± 7	30510 ± 520*	23150 ± 330*
Cl	8.6 ± 1.4	9.9 ± 2.0	7.5 ± 1.3	13 ± 3	8.7 ± 1.5	10 ± 2
I	1.3 ± 0.4	0.75 ± 0.20	2.1 ± 0.6	0.89 ± 0.39	0.26 ± 0.16	1.4 ± 0.6
Na	7.7 ± 1.3	9.4 ± 1.9	6.0 ± 1.2	3.1 ± 0.8	1.6 ± 0.7	2.0 ± 0.7
Mg	0.23 ± 0.06	0.26 ± 0.05	0.25 ± 0.05	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01
P	7.9 ± 1.3	7.5 ± 1.2	12 ± 3	10 ± 2	7.3 ± 0.8	10 ± 2
K	5.9 ± 1.0	9.6 ± 2.7*	11 ± 3*	15 ± 6	28 ± 7*	26 ± 6*
Ca	0.45 ± 0.08	<0.20*	0.28 ± 0.03*	0.23 ± 0.02	<0.20*	<0.20*
Zn	149 ± 16	122 ± 13	187 ± 20	42 ± 5	43 ± 5	54 ± 7
Rye						
Br	33 ± 6	13710 ± 170*	8221 ± 90*	39 ± 8	17820 ± 250*	8935 ± 145*
Cl	14 ± 3	6.3 ± 1.2*	7.1 ± 1.3*	7.4 ± 1.3	6.0 ± 1.0	6.6 ± 1.1
I	0.52 ± 0.06	0.59 ± 0.07	1.0 ± 0.1	0.22 ± 0.03	<0.20	<0.20
Na	6.5 ± 1.3	5.7 ± 1.0	3.3 ± 0.7*	0.38 ± 0.04	0.32 ± 0.03	0.36 ± 0.05
Mg	0.20 ± 0.01	0.33 ± 0.04	0.15 ± 0.01	0.64 ± 0.07	0.87 ± 0.09	0.82 ± 0.08
P	4.7 ± 1.0	5.4 ± 1.2	5.3 ± 1.3	6.5 ± 1.7	5.2 ± 1.0	6.9 ± 2.0
K	6.2 ± 1.1	11 ± 2*	8.2 ± 1.5	15 ± 4	24 ± 5*	18 ± 4
Ca	0.36 ± 0.04	<0.20*	0.35 ± 0.03	0.33 ± 0.04	0.57 ± 0.06	0.43 ± 0.05
Zn	84 ± 9	61 ± 6	65 ± 7	46 ± 5	38 ± 4	40 ± 4
Wheat						
Br	29 ± 4	10080 ± 150*	4573 ± 60*	86 ± 11	10680 ± 160*	5344 ± 70*
Cl	9.3 ± 1.5	7.8 ± 1.3	5.3 ± 1.2*	10 ± 2	9.3 ± 1.6	5.9 ± 1.5*
I	0.55 ± 0.12	1.3 ± 0.5	1.6 ± 0.5	<0.20	<0.20	<0.20
Na	7.2 ± 1.2	1.5 ± 0.3*	4.1 ± 1.0*	0.40 ± 0.05	0.35 ± 0.04	0.28 ± 0.03
Mg	0.30 ± 0.04	0.17 ± 0.02*	0.17 ± 0.02*	0.31 ± 0.04	0.32 ± 0.04	0.17 ± 0.02*
P	3.5 ± 0.9	4.1 ± 1.0	4.3 ± 1.0	6.3 ± 1.1	7.7 ± 1.3	5.1 ± 1.0
K	4.0 ± 0.9	25 ± 3*	5.2 ± 1.0	20 ± 3	41 ± 5*	15 ± 2
Ca	0.48 ± 0.05	0.35 ± 0.03*	0.23 ± 0.03*	<0.20	<0.20	0.21 ± 0.02
Zn	99 ± 10	66 ± 8*	48 ± 6*	37 ± 5	40 ± 4	34 ± 6
Pea						
Br	28 ± 3	6188 ± 70*	2194 ± 30*	4.8 ± 1.2	2972 ± 30*	3796 ± 40*
Cl	3.9 ± 0.5	3.2 ± 0.3	<1*	2.2 ± 0.3	2.3 ± 0.3	1.5 ± 0.2*
I	0.54 ± 0.13	0.85 ± 0.20	0.29 ± 0.20	<0.20	<0.20	0.31 ± 0.09
Na	0.40 ± 0.08	0.62 ± 0.14	0.44 ± 0.09	0.19 ± 0.06	0.09 ± 0.05	0.13 ± 0.05
Mg	0.23 ± 0.03	0.24 ± 0.03	0.34 ± 0.05	0.20 ± 0.02	0.20 ± 0.02	0.27 ± 0.03
P	7.1 ± 1.3	6.6 ± 0.9	7.7 ± 1.2	7.8 ± 2.4	15 ± 5	13 ± 4
K	34 ± 4	39 ± 5	9.1 ± 1.6*	25 ± 3	26 ± 4	30 ± 4
Ca	0.25 ± 0.03	0.35 ± 0.04	<0.20*	0.24 ± 0.04	0.38 ± 0.12	<0.20*
Zn	122 ± 15	102 ± 12	45 ± 5*	58 ± 6	56 ± 6	56 ± 7

*-differences between concentrations of elements in the seedlings germinated in water (no treatments) and in the media spiked with bromides were statistically significant ($P \leq 0.05$).

et al. 1997). On the other hand, the reaction of plants on NH₄Br was different and depended on the plant species. The leaf biomass of rye and wheat seedlings germinated in the NH₄Br-spiked medium was similar to the biomass of the plants not treated with bromides. The biomass of leaves of oat and pea seedlings that were germinated in the medium spiked with NH₄Br decreased statistically significantly ($P \leq 0.05$) compared to the biomass of leaves of the seedlings germinated in the medium without bromides, and the seedlings germinated in the medium spiked with KBr. It may be assumed that the combined exposure to Br⁻ and NH₄⁺ was toxic for these plant species.

Ammonium can affect different physiological and biochemical processes in plants. Under high concentrations, ammonium nutrition can lead to various growth responses including restricted plant growth (Domínguez-Valdivia et al. 2008; Horchani, Hajri and Aschi-Smiti 2011). The symptoms of toxicity, however, differ widely among plant species. It was reported that pea was one of

the most sensitive to NH_4^+ toxicity, especially in terms of its effect on growth rates (Britto and Kronzucker 2002).

Bromine accumulation in different plant species

For our experiments we used four plant species - wheat, rye, oat, and pea. One of the plants, pea, belongs to Dicotyledons, while others are Monocotyledons. It has been speculated that each plant species has developed a specific element composition, and it reflects first of all plant nutrient requirements rather than nutrient availability in the soil (Garten 1978; Markert 1989). One of the reasons for separation of plant species at physiological and biochemical level may be difference in concentrations of macro- and trace elements in a particular plant species. Different plants are capable of maintaining rather stable cytosolic concentrations of nutrients in their tissues that can differ from one to another plant species (Mengel and Kirkby 2001). Therefore, we may expect certain differences in the biochemical processes (including accumulation of Br) occurring in the plants that belong to different botanical taxa. Notice that compared to other selected plant species, more information is available on Br uptake and its phytotoxicity to wheat (Buwalda, Stribley and Tinker 1983; Fransi et al. 1987; Ottman and Pope 2000).

The seedlings germinated in the media spiked with bromides were capable of accumulating high concentrations of Br. The level of Br accumulation, however, depended on the plant species. The highest concentration of Br was in roots and leaves of oat, and the least Br accumulation was in pea (Table 1). These results confirm data of our previous experiments with pea and wheat seedlings grown in the water spiked with KBr. It was shown that the wheat accumulated more Br than the pea (Shtangeeva et al. 2015). The mean Br concentrations in the 7-day-old wheat and pea seedlings grown in the water spiked with 50 mg L^{-1} of Br were 4320 mg kg^{-1} and 1080 mg kg^{-1} in roots and leaves of pea seedlings, respectively and 18200 mg kg^{-1} and 33600 mg kg^{-1} in roots and leaves of wheat seedlings, respectively.

Reported concentrations of Br in the plants growing in soils contaminated with bromides usually do not exceed $1000\text{-}2000 \text{ mg kg}^{-1}$ (Kabata-Pendias 2011). These concentrations are lower than those that we recorded in our experiments. It is known that metals and metalloids may be adsorbed on the surface of the soil particles (Bradl 2004; Violante et al. 2010). Similar effects were observed for bromides (Ahn et al. 2006; Bailey and White 1964). Thus, plants growing in soil are able to absorb less Br than they can absorb from liquid medium where Br is present in easily available form. For example, Br concentrations in plants grown in soils uncontaminated with Br usually do not exceed 40 mg kg^{-1} (Låg and Steinnes 1977; Pourimani et al. 2013; Wishkerman 2006; Yuita et al. 1982), whereas concentration of Br in seaweeds collected from uncontaminated sites may be as high as 2000 mg kg^{-1} (Küpper et al. 2013).

The accumulation of Br also depended on bromides present in the growth medium. All of the plants accumulated more Br if they were germinated in the KBr-spiked medium than in the medium spiked with NH_4Br (with the exception of leaves of pea). Previous experiments (Shtangeeva et al. 2015) showed that in most cases plants accumulate more Br if they are grown in the medium spiked with KBr than if plants are grown in a NaBr-spiked medium.

Effects of different bromides on the concentrations of other elements in plants

The concentration of nutrients in a plant is an important physiological parameter. Our experiments revealed a suppression in the concentrations of several key elements required for the plant growth (K, Na, Ca, Mg, Zn, and Cl) caused by bromides (Table 1). It may be assumed that this limitation in accumulation of the nutrients might result from the bioaccumulation of Br. However, our previous tests (Shtangeeva 2017) showed that Br concentration in young wheat seedlings grown in different liquid media (distilled water, spring water, nutrient solution of

Hoagland) were similar. Besides, the four plant species selected for our experiments had large seeds and could grow for 13 days without supply of nutrients. Nevertheless, there is a chance that the seedlings might be nutrient-deficient to some extent, and this deficiency could affect the uptake of Br and other elements.

In response to the Br bioaccumulation concentration of Cl in roots and leaves of pea and wheat seedlings germinated in the media spiked with NH_4Br decreased statistically significantly ($P \leq 0.05$) compared to Cl content in the seedlings germinated without bromides. Besides, a statistically significant decrease of Cl content occurred in roots of the rye seedlings germinated in the media enriched with different bromides. There were no consequences of Br accumulation on the content of Cl in oat seedlings.

As one might expect, an addition of KBr to the growth medium resulted not only in an increase of Br concentration in plants but also in a statistically significant increase of K content in roots and leaves of all the plants with the exception of pea. In oat, a significant ($P \leq 0.05$) increase of K concentration was also observed in the seedlings germinated in the medium spiked with NH_4Br . On the other hand, concentration of K in roots of the pea seedlings germinated in the medium spiked with NH_4Br decreased statistically significantly ($P \leq 0.05$) compared with K concentration in roots of the plants grown without bromides.

It is interesting to note that the level of increase of Br and K concentrations in the plants exposed to KBr was not the same. It may be assumed that unequal uptake of Br and K could arise from the superimposed effect of two distinct processes, one of which caused the uptake of Br only and another caused an equal uptake of K and Br. Similar effect was described by Steward and Harrison (1939) on absorption of RbBr in potato. Notice that Rb is chemically similar to K. Our previous experiments on effects of KBr on accumulation of Br and K in wheat and pea seedlings grown in water culture also demonstrated higher level of accumulation of Br than K in the plants (Shtangeeva et al. 2015). It was reported (Jackson and Adams 1963) that the cation absorption rates may be independent on the concentrations and rates of absorption of the anions of the external solution, and cations may be absorbed independently on the anions of the solution where plants are grown in exchange with H^+ , while anions are exchanged with OH^- .

In several cases the concentration of Ca in the seedlings germinated in the Br-spiked media was restricted compared to the Ca content in roots and leaves of the plants grown without bromides. The bioaccumulation of Br negatively affected the concentration of Na in roots of wheat. In rye seedlings, a statistically significant ($P \leq 0.05$) suppression of root Na content was observed only if the seedlings were germinated in the medium spiked with NH_4Br . In wheat, different Br compounds also influenced the Zn concentration in roots. In roots of pea, concentration of Zn was statistically significantly ($P \leq 0.05$) lower in the seedlings germinated in the medium spiked with NH_4Br compared to Zn concentration in roots of the seedlings germinated in the medium spiked with KBr and in water not spiked with bromides. Bromides affected concentration of Mg in roots of wheat seedlings. It decreased statistically significantly ($P \leq 0.05$) compared with concentration of Mg in roots of the seedlings germinated without bromides. The suppression of Mg concentration was observed also in leaves of wheat seedlings germinated in the medium spiked with NH_4Br .

Considering that roots were in an immediate contact with bromides it is not surprising that they suffered more than leaves. Besides, different plant species responded differently to an increased concentration of Br in the growth medium. Probably, one of the reasons might be that concentration of one or another element can be different in different plant species (Barker and Pilbeam 2015). In our experiments, more serious consequences were observed for the seedlings germinated in the medium spiked with NH_4Br . It may be hypothesized that this action was due to cumulative effect of Br^- and NH_4^+ . Among other plant species, the most tolerant plant was oat and the most sensitive to bromides was wheat.

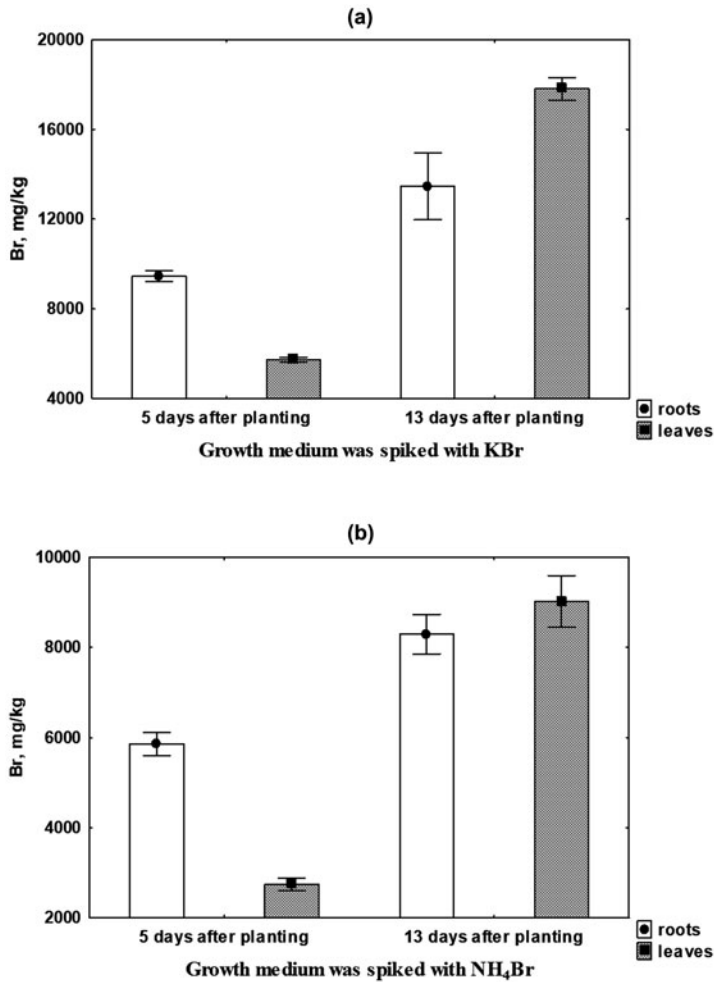


Figure 2. Concentration of Br in roots and leaves of the rye seedlings collected after germination in the media spiked with KBr (a) and NH₄Br (b) during 5 and 13 days. The bars – mean concentration of Br, T - mean ± 0.95 confidence interval.

Temporal variations of Br in plants

The seedlings germinated in the Br-spiked media accumulated more Br with time. After 5 days of germination, all plants accumulated more Br in roots than in leaves. Then, in the 8 days that followed, the Br concentration in leaves of the seedlings germinated in the media spiked with KBr and NH₄Br increased much more than in roots. Typical variations in the Br concentrations are shown in Fig. 2 on the example of rye seedlings. The highest level of Br accumulation was observed when the seedlings were germinated in the medium spiked with KBr. If the seedlings were germinated in the medium spiked with NH₄Br the accumulation of Br was less by almost half. In the course of the experiments, concentration of Br increased in roots and in leaves. However, the increase of Br content in leaves of the seedlings germinated in the media spiked with bromides was greater than in roots with time. It may be suggested that by the end of the experiments concentration of Br in roots has reached possible limit. Roots were unable to protect upper plant parts from translocation of Br. A large part of the trace element was transferred from roots to leaves.

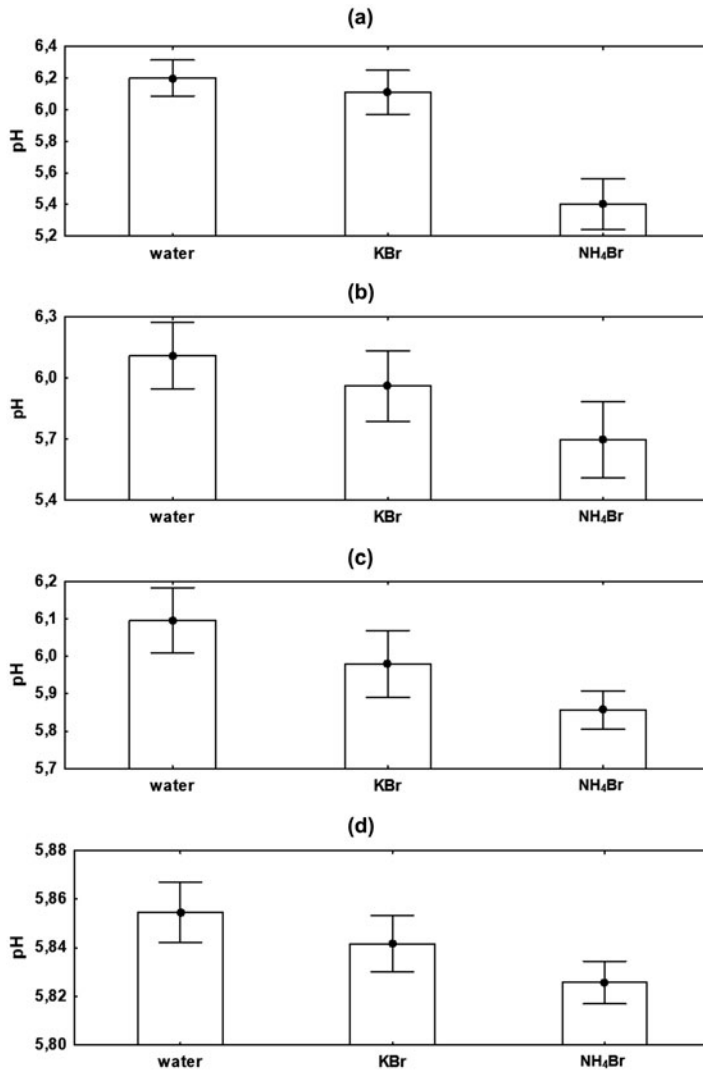


Figure 3. Values of pH of the growth media where oat (a), rye (b), wheat (c), and pea (d) seedlings were germinated in water or in the media spiked with KBr and NH₄Br. The bars – mean pH values, T - mean ± 0.95 confidence interval.

Variations in the pH of the growth media

By the end of the experiments the pH values of the growth media were similar for untreated and KBr-spiked media (Fig. 3). The pH values of the media that were spiked with bromide of ammonium were statistically significantly ($P \leq 0.05$) lower compared to the pH of the medium not spiked with bromides. Probably, this phenomenon might be due to the fact that ammonium ion is a weak acid. Besides, the pH of the media where pea seedlings were germinated was lower than the pH of the media with other plants. It may be suggested that root exudates of pea released to the surrounding medium more acid components than other plant species. Marked changes in the pH of the culture media also may be due to unequal absorption of cations and anions (Jacobson et al. 1957; Hribar et al. 2002).

Correlation between elements in plants

Results of correlation analysis demonstrated an excellent correlation between concentrations of Br in roots and in leaves ($r = 0.84$, $P \leq 0.05$). A statistically significant ($P \leq 0.05$) positive correlation between concentrations of elements in roots and leaves occurred for Cl ($r = 0.79$), Na ($r = 0.67$), Zn ($r = 0.47$), K ($r = 0.41$), and P ($r = 0.39$).

Calculation of correlation coefficients between Br and other elements in different plant parts demonstrated a statistically significant positive correlation between Br and Na ($r = 0.43$) in roots. In leaves, a statistically significant positive correlation was observed between Br and Cl ($r = 0.48$) and between Br and K ($r = 0.54$).

Conclusion

Plants were capable of accumulating rather high concentrations of Br if they were grown in the aqueous media enriched with different bromides. The accumulation of Br negatively affected the plant biomass and also resulted in a decrease of concentrations of nutrients in the plants. The most significant negative effects were caused by ammonium bromide. Oat was the most tolerant and wheat was the most sensitive to the bromide actions.

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