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RESEARCH PAPERS

Effects of Potassium and Sodium Bromides on *Triticum aestivum* and *Pisum sativum*[1]

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Abstract—Different bromine compounds have found numerous applications. Nevertheless, there is still insufficient information about environmental chemistry of this trace element. In our research, a greenhouse pot experiment was carried out with the following aims: (1) to study the response of wheat and pea seedlings to an increase of concentrations two bromides (KBr and NaBr) in soil, (2) to assess the influence of root exudates of the two plant species on uptake of Br and some other macro- and trace elements when wheat and pea grow close to each other or far apart, and (3) to estimate possible allelopathic effects on the uptake of different elements by plants and also on the concentrations of polycyclic aromatic hydrocarbons in uncontaminated soil and in the soil contaminated by bromides. Both plant species were capable of accumulating large amounts of bromine. The concentration of bromine in roots of pea was higher than in wheat roots, and was always higher in leaves of wheat than in leaves of pea. More bromine was accumulated in roots of wheat and pea seedlings growing separately than in roots of the seedlings grown close to each other. Growth of wheat and pea seedlings in uncontaminated and spiked with bromides soils resulted in decrease of the concentrations of some polycyclic aromatic hydrocarbons in the rhizosphere soil of the plants. The variations depended on the plant species and were different when the plants were grown close to each other and separately.

Keywords: *Triticum aestivum*, *Pisum sativum*, wheat, pea, bromine, root exudates, polycyclic aromatic hydrocarbons

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INTRODUCTION

Bromine (Br) is a very interesting trace element that has found wide applications. Various Br compounds are used in industry [1, 2], agriculture [3, 4], and medicine [5, 6]. Although rather much experimental data on concentration of Br in some important crops have already been collected, the information about its distribution in soil used for agricultural production and in different crops is not enough to make final inference about benefits or possible phytotoxicity of this trace element for various plant species. Besides, results of determining Br in the same plant species can often vary. This may in part be due to the wide variety of sampling procedures used by different scientists. In addition, the determination of Br, a volatile trace element, can be difficult [7, 8].

Plant roots release various organic compounds into the surrounding medium. Each plant species releases its specific root exudates into the rhizosphere [9]. These organic compounds can have both beneficial and harmful effects on plants [10]. In nature, the plants usually grow close to each other and thus, root exudates of one plant can interact with exudates of another plant species. It is possible that a new combination of organic compounds may have different effects on the rhizosphere of plants growing close and far from each other. Thus, one of the important points that we would like to consider is the effect of root exudates on the Br uptake by plants in the case when two different plant species grow separately or in close proximity to each other.

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment [11]. They are rather stable and can persist in soil for several years [12]. The sources of PAHs are incomplete combustion of organic matter. The PAHs can also be produced when organic sediments are chemically transformed into fossil fuels such as oil and coal. Therefore, natural PAHs originate mostly from volcanic eruption, plant emissions, and fires [13]. The PAHs are capable of changing physicochemical properties of the soil, thus affecting the soil inhabitants and plants. The PAHs are

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[1] The text was submitted by the authors in English.

usually present in unpolluted soils at rather low concentrations [14, 15]. Nevertheless, their influence on the soil and plants can be quite strong. Some information about interactions between different metals and PAHs in soil has been published [16–18]. Up to now, however, not much work has been done to examine the interactions between Br and PAHs in the rhizosphere soil.

The main purposes of the experimental work were (1) to study the response of wheat and pea seedlings to an increase in the concentrations of two bromides (KBr and NaBr) in soil; (2) to assess the influence of root exudates of the two plant species on uptake of Br and some other macro- and trace elements when wheat and pea grow close to each other or far apart; (3) to estimate possible allelopathic effects on the uptake of different elements by plants and also on the concentrations of PAHs in uncontaminated soil and in the soil contaminated by bromides.

MATERIALS AND METHODS

Plant material and experimental design. Wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) seeds were germinated on a wet filter paper for 7 days. Uniformed seven-day-old germinated seedlings were transferred to pots (20 seedlings in a pot) filled with soil (6 kg of soil in a pot). The soil had a loamy sand texture (sand 74%, silt 24%, clay 2%). Before planting, the soil was exposed to solutions of bromides (100 mg of NaBr or 100 mg of KBr per kg of soil), or the same amount of ordinary water (control) was added to the soil. Wheat and pea seedlings were grown either in separate pots, or both the seedlings were grown together in the same pot under the same conditions in the soil that was exposed to ordinary water or to solutions of KBr or NaBr. The temperature in a naturally illuminated greenhouse was 25°C during the day and 22°C at night. The soil pH (1 : 2.5 H_2O) was measured. A completely randomized design was used with three replicates. Plants and soil (adhering to the plant roots) were collected within 10 days after transfer of the seedlings to soil. Plants were washed carefully by tap water. Roots were separated from leaves and then the soil and plant samples were air-dried at room temperature to a constant weight.

Preparation of samples for elemental analysis. Concentrations of Na, Mg, P, Cl, K, Ca, Mn, Cu, Zn, and Br in the plant and soil samples were determined after leaching the samples with tetramethyl ammonium hydroxide (TMAH) at mild temperature using the method described by Tagami et al. [19]. The samples (approximately 100 mg of each sample) were placed into the Teflon® PFA Advanced Composite Vessels (volume of 100 mL, CEM Corp.). Two mL of TMAH solution were added; the vessels were closed and heated in a sand bath at 60°C for 16 h. Then the samples were diluted to 25 mL with ultrapure water. For ICP-MS determination, samples were diluted to 1 : 2 with UP-H_2O. For ICP-OES determination, samples were diluted to 1 : 2 with 5% (v/v) HNO_3.

Elemental analysis. Perkin Elmer Optima 5300 DV ICP-OES (PerkinElmer, United States) with axial viewing of the plasma was used for determination of Na, Mg, P, K, Ca, Mn, Cu, and Zn. The ICP-OES instrument was equipped with an AS-93plus auto sampler, a Ryton double-pass Scott-type spray chamber, and the Gem Tip Cross-flow pneumatic nebulizer. Thermo Elemental X7 quadrupole ICP-MS (Thermo Elemental, United Kingdom) was used for the determination of Cl and Br. The ICP-MS instrument was equipped with a standard low-volume glass impact bead spray chamber (Peltier cooled at 3°C), a concentric glass nebulizer, and a Cetac ASX-500 autosampler (Cetac Technologies, United States).

Quality control. The accuracy of the analytical procedure was evaluated by analyzing certified reference material (CRM) NIST SRM 1573a (Tomato leaves). The results were in a good agreement with the certified and informative values. This means that the elements were successfully extracted from the CRM by using TMAH extraction at 60°C.

Analysis of organic compounds in soil. The method of extracting essential oils from terrestrial and aquatic plants [20, 21] was applied to extract volatile organic compounds from the soil samples. For the steam distillation process on the Clevenger apparatus, 2 g of each soil sample were prepared. The steam hydrodistillation procedure lasted for 6 hours. Extraction into hexane was carried out directly during the process of hydrodistillation. The obtained extracts (5 mL volume) were stored in a freezer at –18°C. The analysis was performed on a TRACE ISQ gas chromatograph-mass spectrometer (Thermo Electron Corporation, USA) equipped with a quadrupole mass analyzer and Thermo TG-SQC Column (15 m, inner diameter 0.25 mm and 0.25 mm film). The ionization voltage constituted 70 eV, and helium served as a carrier gas. The scanning mode was used for the entire mass range (30–580 amu). Mass spectra were recorded in a programmed temperature mode (35°, 3 min; 2°/min to 60°, 3 min; 2°/min to 80°, 3 min; 4°/min to 120°, 3 min; 5°/min to 150°, 3 min, and 15°/min to 240°, 10 min). The identification of the organic compounds was performed in the course of a step-by-step analysis of chromatograms. The NIST-2014 and Wiley mass spectrum libraries were applied to identify the detected compounds. Retention indices calculated as described by Zellner et al. [22] were used to refine the identification. Quantitative analysis was conducted with decafluorobenzophenone and benzophenone (Merck certified reference materials CAS Numbers 119-61-9 and 853-30-4) as internal standards.

Data analysis. For multivariate statistical analysis, Statistica for Windows 6.0 Software packages (StatSoft, United States) were used. Mean concentrations of elements were calculated. Analysis of variances was carried out to assess possible differences between the samples. Before the analysis was done, the Shapiro–Wilk test was applied to assess the normality of the data. Additionally, cluster analysis (CA) was performed to study similarities and distinctions between the experimental samples. For the CA, Ward's method was applied using the squared Euclidean distances as a measure of similarity.

RESULTS AND DISCUSSION

Effect of Root Exudates of Wheat and Pea Seedlings on Soil pH

Compared to initial soil, the pH of the soil taken from roots of wheat and pea seedlings was statistically significantly ($P < 0.001$) higher (Fig. 1). Relative to the pH of the rhizosphere soil of the plants grown separately, the increase of the soil pH values was larger ($P < 0.05$) when the plants were grown close to each other. The only exception was the pH of the soils taken from roots of the wheat seedlings grown in uncontaminated soil in different pots and together. In this case the pH values of the soils were similar. The least influence of the treatments on the pH of the rhizosphere soil was observed when wheat and pea seedlings were grown separately from each other in the soil spiked with KBr.

The effect of root exudation on the pH of the rhizosphere soil is expectable. The root exudates consist of various compounds that can affect the soil acidity. The composition of the root exudates of different plant species is usually distinct. Besides, it can vary depending on many environmental factors [23, 24]. In particular, the response of neighboring plants to changes in the environmental conditions can differ depending on whether the plants belong to the same or different species [25]. It was reported that the less the phylogenetic relationship of plants, the greater the difference in the composition of their exudates [26].

Bromine Accumulation in Wheat and Pea Seedlings

Both plant species were capable of accumulating rather large amounts of Br (Fig. 2), especially when the plants were grown in the soil contaminated by KBr. Roots of the wheat and pea seedlings grown separately accumulated more Br than roots of the seedlings grown close to each other. The concentration of Br in roots of pea was higher than in wheat roots. Bromine was also easily transferred from roots to upper plant parts. The Br concentration was always higher in leaves of wheat than in leaves of pea.

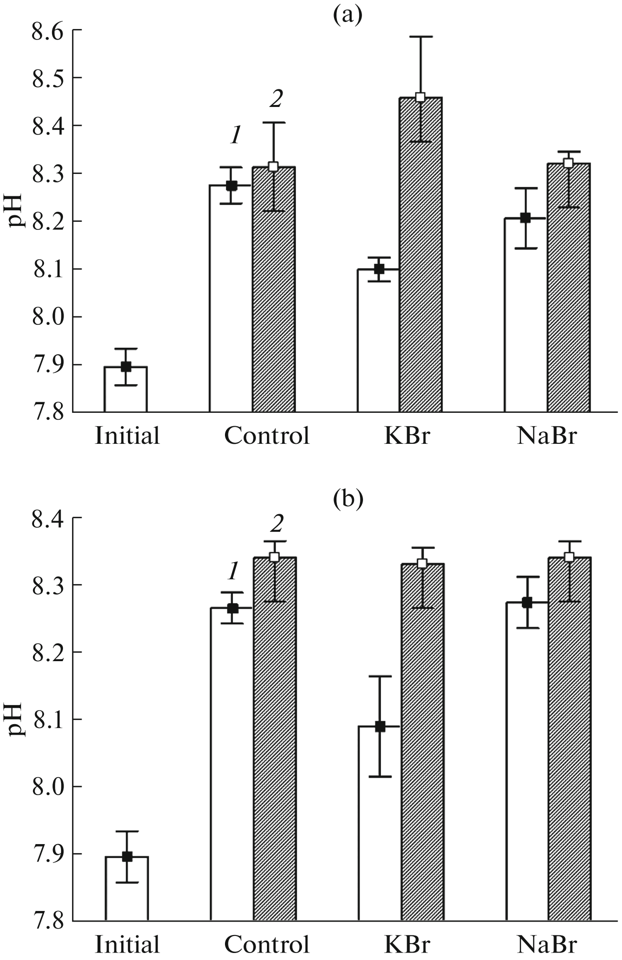

Fig. 1. The pH values of initial soil and soil taken from roots of wheat (a) and pea (b) grown in uncontaminated soil (Control) and in the soil spiked with KBr or NaBr. Plants were grown separately (*1*) and close to each other (*2*).

Different concentrations of some elements in wheat and pea grown under the same conditions have been reported by other researchers. Froese et al. [27] compared uptake of P, Fe, and Zn by the plants and found rather serious differences between concentrations of the elements in wheat and pea. Baruah et al. [28] showed that wheat was capable of accumulating larger amounts of Cu, Cd, and Pb than pea. The differences in the ability of wheat and pea to uptake different amounts of elements from the same growth medium may be due to the fact that, according to botanical classification, these plant species belong to different clades: wheat is monocot and pea is eudicot. As a rule, there is no identical relationship between the concentration of a particular element in soil and its concentration in various plant species growing in the soil. The accumulation abilities of plants are different.

The accumulation of Br in wheat and pea seedlings resulted in a decrease of the Br concentration in the rhizosphere soil of both plant species (Fig. 3). More severe changes were found in the soil taken from roots of the plants growing close to each other.

Differences in the Element Uptake by the Plants Growing Separately and Close to Each Other

Table 1 shows mean concentrations of elements in wheat and pea seedlings grown in uncontaminated soil

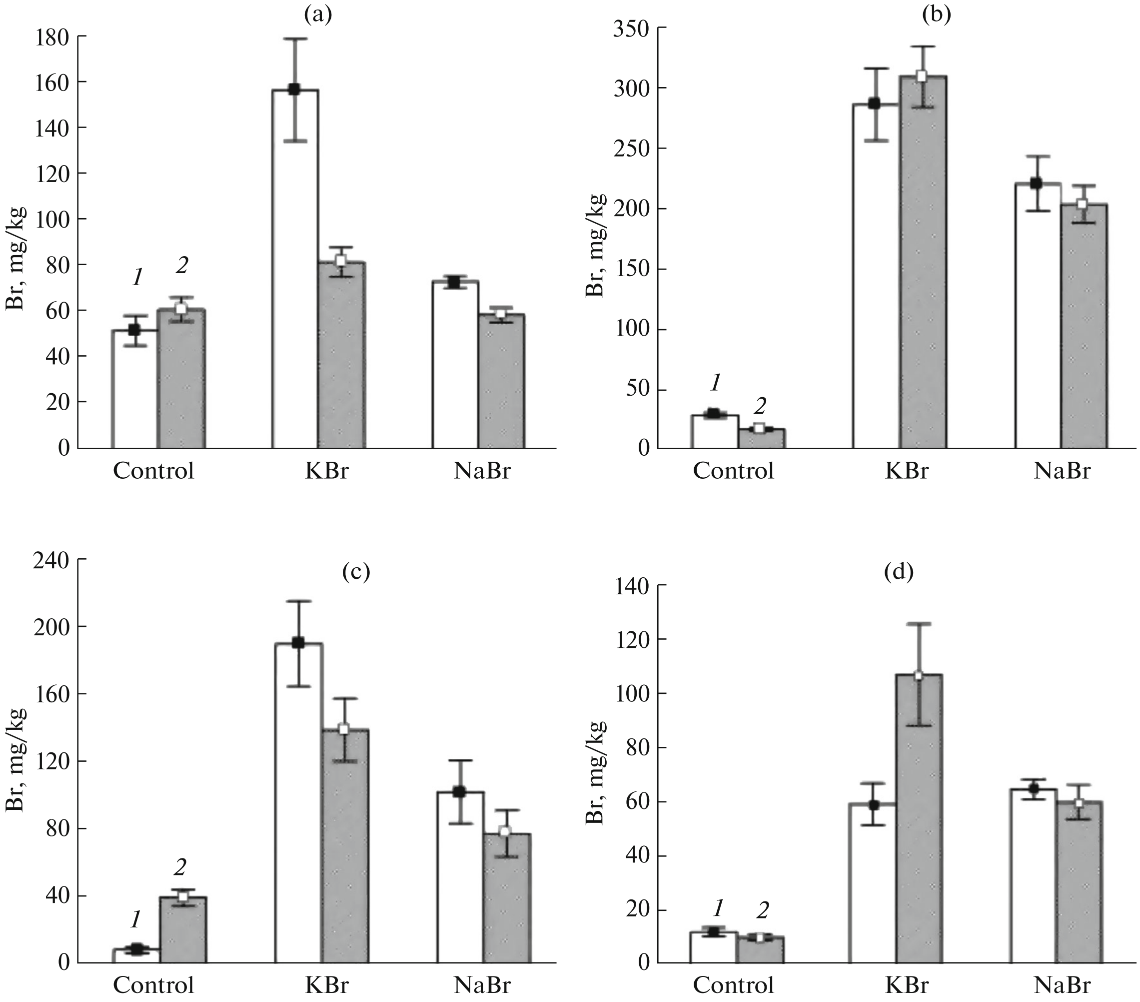

Fig. 2. Concentration of Br in roots (a) and leaves (b) of wheat seedlings and in roots (c) and leaves (d) of pea seedlings grown in uncontaminated soil (Control) and in the soil spiked with KBr or NaBr. Plants were grown separately (*1*) and close to each other (*2*).

separately and close to each other. In roots of both plant species growing in close proximity, the concentration of Mn was higher ($P < 0.01$) than in roots of the plants growing in different pots. In leaves of the wheat seedlings grown separately from the seedlings of pea, the concentration of Na was statistically significantly ($P < 0.01$) lower compared with concentration of the element in leaves of the wheat seedlings grown close to the pea seedlings. The differences between concentrations of elements in leaves of the pea seedlings grown together and separately were statistically insignificant.

The calculation of ratios of the concentration of a particular element in leaves to the concentration of the element in roots showed that in the wheat and pea seedlings grown together the leaf to root ratios of Mn were lower as compared to the ratios in the plants grown separately. More significant (3.8 times) decrease was observed for pea. For wheat, the decrease was 2.3 times. The ratios of other elements in the plants grown separately and together were similar.

Ayres and Thornton [29] reported that wheat and pea grown under the same conditions in solution culture showed a different nature of the amino acids released by their roots. Kovalova et al. [30] studied the composition of root exudates of wheat and pea seedlings and found that exudates of wheat seedlings had a high (50–67%) content of carbohydrates, while the main (80–85%) components of exudates of pea seedlings were proteins. It can be suggested that in the case when wheat and pea grow close to each other, the new combined composition of root exudates of the plants could affect the uptake of Mn by roots of wheat and pea seedlings and also influence the translocation of Na from roots to leaves of wheat seedlings.

Results of Multivariate Statistical Analysis of Experimental Samples

Cluster analysis based on the concentrations of elements in the plant samples showed that roots of pea seedlings were well separated from roots of wheat

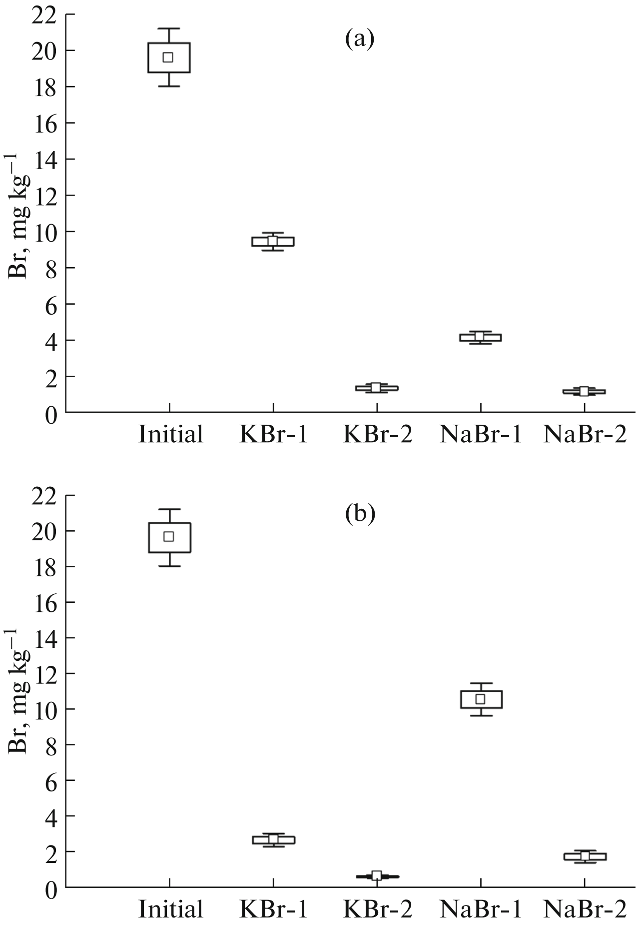

Fig. 3. Concentration of Br in initial soil and in the soil taken from roots of wheat (a) and pea (b) seedlings grown in the soil spiked with KBr or NaBr. Plants were grown separately (*1*) and close to each other (*2*).

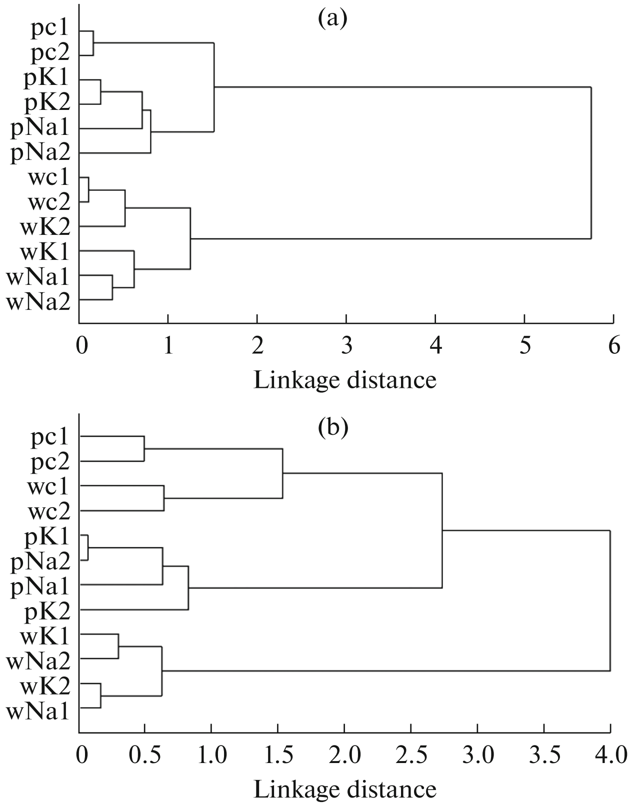

Fig. 4. Cluster analysis (Ward's method) of roots (a) and leaves (b) of wheat (w) and pea (p) seedlings grown in clean (uncontaminated) soil (c) and in soil spiked with KBr (K) or NaBr (Na). Plants were grown separately (*1*) and close to each other (*2*).

seedlings (Fig. 4). Within each group, roots of the seedlings grown in uncontaminated soil were separated from roots of the seedlings grown in the soil spiked with bromides. However, there were no clearly seen differences between roots of the plants grown separately and close to each other. Leaves were separated into three groups: (1) leaves of the plants grown in uncontaminated soil (within the group, leaves of wheat and pea seedlings were separated from each other); (2) leaves of pea grown in the Br-contaminated soil; (3) leaves of wheat grown in the Br-contaminated soil. No real separation of leaves of the plants grown close to each other and separately was found.

Effect of Bromides on Concentrations of PAHs in the Rhizosphere Soil of Wheat and Pea Seedlings Grown Separately and Close to Each Other

Figure 5 illustrates the variations in the concentrations of some PAHs in soil. Growth of pea and wheat seedlings during 10 days in uncontaminated and bromide-contaminated soils led to certain changes in the concentrations of phenanthrene, fluoranthene, pyrene, and naphthacene in the rhizosphere soil of the plants. The variations depended on the plant species and were different when the plants were grown close to each other and separately.

When wheat and pea seedlings were grown separately, in most cases the concentrations of the PAHs in the rhizosphere soil of both the plants decreased as compared to the concentrations of phenanthrene, fluoranthene, pyrene, and naphthacene in initial soil. The largest decrease was observed in the soil taken from roots of pea seedlings, while concentrations of the PAHs in the soil taken from roots of the wheat seedlings grown in the soil spiked with NaBr either did not change or even increased.

When wheat and pea seedlings were grown close to each other, in most cases the concentrations of phenanthrene, fluoranthene, pyrene, and naphthacene in the soil taken from roots of pea slightly decreased as compared to those in initial soil. In the soil taken from roots of wheat, the decrease of the PAHs was also observed, and the most considerable changes were often found in the rhizosphere soil of the

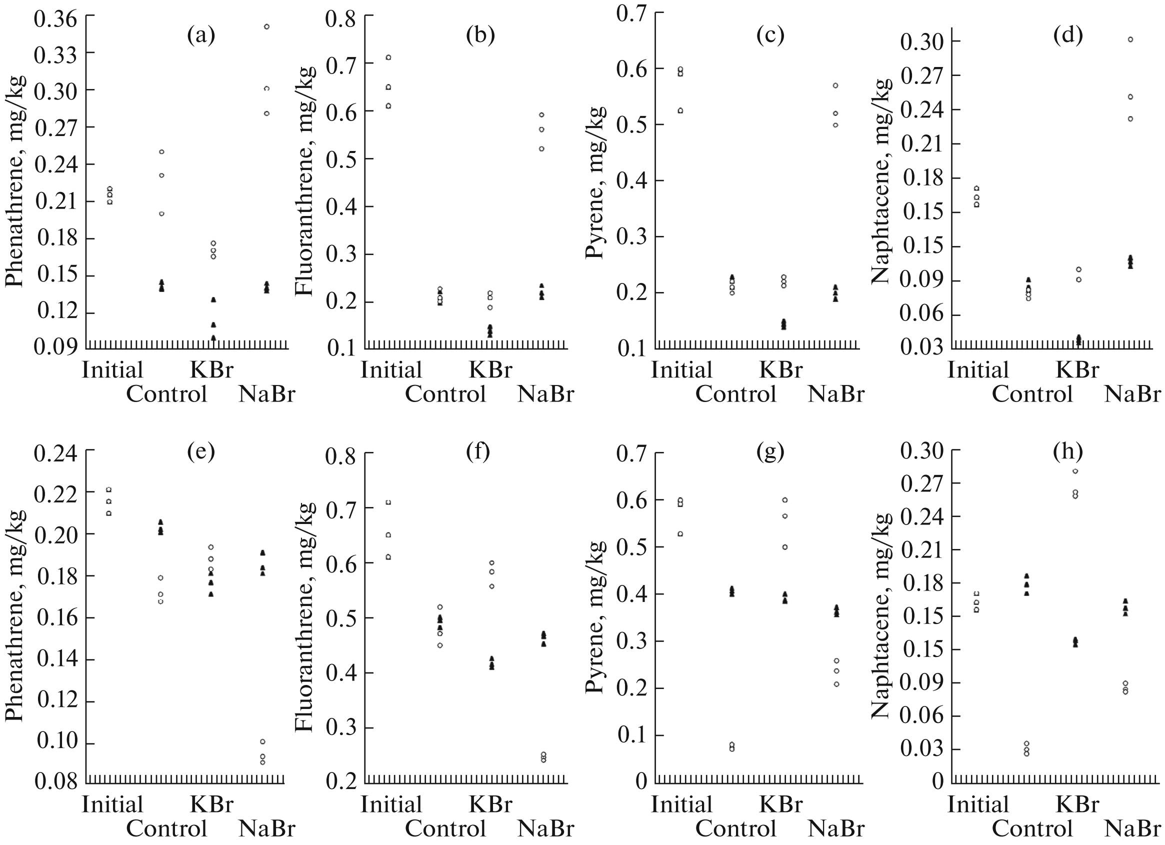

Fig. 5. Concentrations of phenanthrene (a, e), fluoranthene (b, f), pyrene (c, g), and naphthacene (d, h) in the rhizosphere soil of pea (triangles) and wheat (circles) seedlings grown in uncontaminated soil (Control) and in the soil spiked with KBr or NaBr. Plants were grown separately (a–d) and close to each other (e–f).

Table 1. Concentrations of elements in wheat and pea seedlings grown in uncontaminated soil separately (1) and close to each other (2)

Elements	Wheat		Pea	
	1	2	1	2
		Roots		
Na, %	0.91 ± 0.25	0.91 ± 0.19	1.60 ± 0.26	1.47 ± 0.05
Mg,%	0.14 ± 0.02	0.16 ± 0.04	0.17 ± 0.11	0.17 ± 0.03
P, %	0.09 ± 0.01	0.10 ± 0,01	0.29 ± 0.05	0.31 ± 0.01
Cl, %	0.73 ± 0.02	0.81 ± 0.22	1.66 ± 0.33	1.83 ± 0.13
K, %	0.80 ± 0.17	0.90 ± 0.32	3.91 ± 0.27	4.43 ± 0.83
Ca, %	0.20 ± 0.05	0.12 ± 0.01	0.20 ± 0.07	0.17 ± 0.04
Mn, mg/kg	2.40 ± 0.05*	4.43 ± 0.36	0.60 ± 0.27*	3.01 ± 0.87
Cu, mg/kg	44 ± 9	38 ± 10	24 ± 1	26 ± 4
Zn, mg/kg	44 ± 13	57 ± 7	54 ± 17	55 ± 11
		Leaves		
Na, %	0.96 ± 0.08*	1.52 ± 0.09	0.15 ± 0.02	0.18 ± 0.03
Mg,%	0.02 ± 0.01	0.02 ± 0.01	0.16 ± 0.03	0.19 ± 0.06
P, %	0.51 ± 0.05	0.41 ± 0.08	0.56 ± 0.10	0.64 ± 0.08
Cl, %	2.29 ± 0.41	2.73 ± 0.69	0.85 ± 0.02	0.76 ± 0.05
K, %	4.31 ± 0.13	3.45 ± 0.87	2.91 ± 0.25	2.91 ± 0.40
Ca, %	0.10 ± 0.04	0.08 ± 0.03	0.13 ± 0.04	0.17 ± 0.03
Mn, mg/kg	3.92 ± 1.12	3.22 ± 0.92	5.02 ± 1.89	6.69 ± 2.01
Cu, mg/kg	10 ± 2	6.64 ± 2.15	9.16 ± 1.96	7.71 ± 2.77
Zn, mg/kg	42 ± 6	37 ± 1	59 ± 18	71 ± 29

Values are means ± standard deviation of one experiment with three biological replicates. Asterisk indicates statistically significant ($P < 0.01$) differences between plants grown separately and close to each other.

wheat seedlings grown in the soil spiked with NaBr. This was quite opposite of the effect observed when wheat and pea were grown separately. This indicates a real reaction to the interactions between various compounds released into the rhizosphere by wheat and pea seedlings.

The ability of plants to degrade PAHs in the rhizosphere soil is well-known [31, 32]. The decrease in the concentrations of different PAHs in the vegetated soils was demonstrated on the example of various plant species. An important point is that different plants are capable of degrading certain PAHs differently [33]. It should be mentioned, however, that the experimental results describing the PAH degradation in soil are often contradictory [34]. On the one hand, it was shown that root exudates can stimulate the decomposition of some PAHs, but on the other, there might be a situation when the exudates stimulate an accumulation of PAHs in the rhizosphere zone.

CONCLUSIONS

Our experiment showed that wheat and pea seedlings are able to accumulate high concentrations of Br when grown in the soil contaminated with different bromides. The highest Br accumulation was observed in the plants grown in the soil spiked with KBr. The accumulation capacity depended on the plant species and was different for the plants grown close to each other and separately. Wheat and pea seedlings were capable of significantly reducing the concentration of some PAHs in the rhizosphere soil of the plants. The variations were also different for the two plant species and depended on the distance between wheat and pea as well as on the soil contamination by different bromides. It can be concluded that root exudates of wheat and pea seedlings can cause different plant responses if they grow close to each other or separately.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving humans or animals performed by any of the authors.

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