

## A STUDY ON THE FLUORIDE CONTENT AND THE ENZYMATIC ACTIVITY IN SOIL EXPOSED TO INORGANIC AMMONIUM SALT AND QUATERNARY AMMONIUM SALTS WITH HEXAFLUOROPHOSPHATE ANIONS

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**ABSTRACT:** This study examines the effect in sandy soil on the content of two fluoride forms, which are soluble (extracted with  $\text{CaCl}_2$ ) and potentially available to plants (extracted with  $\text{HClO}_4$ ), and the activity of dehydrogenases, acid phosphatase, alkaline phosphatase, and *o*-diphenol oxidase of three quaternary ammonium salts (QAS) with the  $[\text{PF}_6]^-$  anion (tetramethylammonium hexafluorophosphate  $[\text{TMA}][\text{PF}_6]$ , tetrabutylammonium hexafluorophosphate  $[\text{TBA}][\text{PF}_6]$ , and tetrahexylammonium hexafluorophosphate  $[\text{THA}][\text{PF}_6]$ ) and the inorganic salt ammonium hexafluorophosphate. The results showed an increase in fluoride content, especially in the form potentially available to plants ( $\text{F-HClO}_4$ ). An inhibition of dehydrogenases, acid phosphatase, and *o*-diphenol oxidase was observed, while the alkaline phosphatase was stimulated. The highest effect was found after treatment with the highest dosages of QAS. The fluoride content was negatively correlated with the activity of the dehydrogenases, acid phosphatase, and *o*-diphenol oxidase, and was positively correlated with alkaline phosphatase activity.

Keywords: Enzymatic activity; Fluoride; Quaternary ammonium salts; Soil.

### INTRODUCTION

Quaternary ammonium salts (QAS), constitute a huge group of substances which, due to their desirable physical and chemical properties, still attract great interest in many industrial sectors. An increased concentration of these compounds in the environment may lead to the contamination of the natural environment.<sup>1</sup> QAS have been known for a long time for their supremely desirable froth-like, moisturizing, emulsifying, surfactant, antielectrostatic, preserving, algicide, antifungal, and bactericidal properties resulting in a worldwide QAS production of one million tons at the end of the twentieth century.<sup>2</sup> Most of the uses of these chemicals lead to their release into soil and water systems. In underdeveloped and developing countries, where sewage systems are poor, the household waste water is released directly into the soil or into water streams without adequate treatment.<sup>3</sup>

QAS are usually toxic to microorganisms. These compounds are generally more toxic to Gram-negative than to Gram-positive soil microorganisms and spore formation is one of the survival mechanisms for microorganisms to overcome stress in an aqueous environment.<sup>4</sup> Also, many QAS contain anions with fluoride atoms, which could hydrolyze in the environment.<sup>5</sup>

Despite being an anion, fluoride is immobile in soil. Saeki<sup>6</sup> studied the adsorption sequences of toxic inorganic anions in soil and found that fluoride was the species

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retained with highest affinity. The main factors that influenced mobility of fluoride is pH and the formation of aluminum and calcium complexes.<sup>7</sup>

Stress caused by changes in the soil environment due to the presence of foreign chemicals, e.g., fluoride can be assessed in advance through sensitive soil quality parameters.<sup>8</sup> Enzymatic activity is considered as a sensitive indicator of environmental disturbances in soils caused by the presence of foreign chemicals,<sup>9</sup> such as QAS, but information on the influence of QAS on the microbial and enzymatic activities in soil is rarely available in the literature.

Dehydrogenases belong to one of the most important classes of enzymes, because they occur in all living microorganism cells.<sup>10</sup> As they are closely connected with microbiological redox processes, dehydrogenases are often considered as an index of the general microbiological activity of the soil.<sup>11</sup> The significance of dehydrogenases as a pollution indicator is additionally supported by their lack of ability to accumulate in the extracellular environment. The role of dehydrogenases consists of the biological oxidation of organic matter in the soil by hydrogen transfer from the organic substrate to inorganic acceptors.<sup>12</sup> The *o*-diphenol oxidase, and other phenol oxidases, mediate key soil ecosystem functions of lignin degradation, humification, carbon mineralization, and dissolved organic carbon export.<sup>13</sup> Moreover, Floch et al. reported that activity of these enzymes may to be used as a bioindicator of the presence of xenobiotic compounds, such as aromatic pollutants.<sup>14</sup> Phosphatases are very important in plant nutrition and in organic P mineralization in soils.<sup>15</sup> They catalyse the hydrolytic cleavage of the C-O-P ester bond of organic P present in soil, releasing inorganic P for plant use, and thus play a fundamental role in P cycling.<sup>16</sup> Therefore, the main goal of this paper is to compare the effect of three different QAS and ammonium hexafluorophosphate with the hexafluorophosphate anion on fluoride that is soluble (extracted with CaCl<sub>2</sub>) and potentially available to plants (extracted with HClO<sub>4</sub>), and the activity of dehydrogenases, phosphatases and *o*-diphenol oxidase in sandy soil.

## MATERIALS AND METHODS

The QAS used in the experiment were tetramethylammonium hexafluorophosphate [TMA][PF<sub>6</sub>], tetrabutylammonium hexafluorophosphate [TBA][PF<sub>6</sub>], tetrahexylammonium hexafluorophosphate [THA][PF<sub>6</sub>], and the inorganic salt ammonium hexafluorophosphate [A][PF<sub>6</sub>].

The pot experiment was carried out in the vegetation hall of the Department of Biochemistry and Ecotoxicology at Jan Długosz University in Częstochowa. A monocotyledonous plant, the spring barley (*Hordeum vulgare* L.) and a dicotyledonous plant, the common radish (*Raphanus sativus* L. subvar. radicula Pers.) were used in the experiment. The seeds of the plants, originating from the same source, were sown into a 90 mm-diameter plastic plant pot that was filled with the reference soil and a soil thoroughly mixed with the studied QAS and ammonium hexafluorophosphate. The dosages of these that were used were 0, 1, 10, 50, 100, 400, 700, and 1000 mg/kg dry matter (DM). The amount of fluoride applied to soil with these compounds is presented in Table 1. The soil used in the experiment was loamy sand containing approximately 10% of dissolved matter and an organic carbon content of 9.0 g/kg. The pH in 1 M KCl was equal to 6.0. Throughout the testing

period of 14 days, a constant substrate moisture content at the level required for the plants (70% of the field water capacity), a constant temperature,  $20 \pm 2^\circ\text{C}$ , a constant illumination of  $170 \mu\text{mol}/\text{m}^2/\text{sec}$  for 16 hr a day, and darkness for 8 hr/day were maintained in the system.

On day 14 the soil samples were collected to analyze for fluoride content and enzymatic activities. The fluoride content extracted with 0.01 M  $\text{CaCl}_2$  (water-soluble)<sup>17</sup> and extracted with 2 M  $\text{HClO}_4$  (potentially available to plants)<sup>18</sup> were determined in the presence of total ionic strength adjustment buffer TISAB III using the potentiometric method with an Orion Research ion-selective electrode. Activity of dehydrogenases [EC 1.1.1.x]<sup>19</sup>, acid phosphatase [EC 3.1.3.2], alkaline phosphatase [EC 3.1.3.1]<sup>20</sup>, and *o*-diphenol oxidase [EC 1.10.3.1]<sup>21</sup> were measured using spectrophotometric methods with Shimadzu UV-VIS 1800 equipment.

The results were submitted to a factorial analysis of variance (ANOVA) using the program STATISTICA 12.5 (StatSoft Inc.). The one-way ANOVA were employed to analyze the fluoride contents and the enzyme activities. The least significant differences were calculated using Tukey's test. Values of *p* lower than 0.05 were considered as evidence for statistical significance. Pearson's correlation coefficients between fluoride contents and enzyme activities were calculated as well.

**Table 1.** Amount of fluoride applied to the soil containing one of three quaternary ammonium salts (QAS) (tetramethylammonium hexafluorophosphate [TMA][PF<sub>6</sub>], tetrabutylammonium hexafluorophosphate [TBA][PF<sub>6</sub>], and tetrahexylammonium hexafluorophosphate [THA][PF<sub>6</sub>]) and ammonium hexafluorophosphate [A][PF<sub>6</sub>]

Dosage of QAS and [A][PF <sub>6</sub> ] applied to the soil (mg/kg dry matter)	Amount of fluoride in the QAS and [A][PF <sub>6</sub> ] dosage applied to the soil (mg F/kg dry matter)			
	[A][PF <sub>6</sub> ]	[TMA][PF <sub>6</sub> ]	[TBA][PF <sub>6</sub> ]	[THA][PF <sub>6</sub> ]
1	0.70	0.52	0.29	0.23
10	6.99	5.20	2.94	2.28
50	34.97	26.01	14.71	11.41
100	69.94	52.03	29.42	22.82
400	279.75	208.11	117.70	91.27
700	489.57	364.20	205.97	159.71
1000	699.39	520.29	294.25	228.16

## RESULTS AND DISCUSSION

The range of the soluble fluoride content in the soil, extracted with 0.01 M  $\text{CaCl}_2$  (F- $\text{CaCl}_2$ ), was 0.96–1.30 mg/kg dry matter (DM). The application of all concentrations of [A][PF<sub>6</sub>] did not result in significant changes of the F- $\text{CaCl}_2$  content in soil, collected from spring barley as well as common radish. However, after addition of all the QAS, a gradual increase of the F- $\text{CaCl}_2$  content was observed, with the increment of QAS dosage (Table 2). In the soil from spring barley, the addition of all the QAS at the dosage of 1 mg/kg DM did not have a significant effect on F- $\text{CaCl}_2$ , whereas in the soil from common radish, significant F- $\text{CaCl}_2$  changes

were not reported for [TMA][PF<sub>6</sub>] at the dosage of 1 mg/kg DM, for [TBA][PF<sub>6</sub>] at the dosages of 1 mg/kg and 10 mg/kg DM and for [THA][PF<sub>6</sub>] at the dosages of 1, 10, and 50 mg/kg DM. Compared to the control soil, the greatest increase of F-CaCl<sub>2</sub> content in soil from spring barley, after the application of [TMA][PF<sub>6</sub>] was determined for the dosage of 400 mg/kg DM (73.98%), whereas for [TBA][PF<sub>6</sub>] and [THA][PF<sub>6</sub>] it was for the dosage of 700 mg/kg DM (122.22% and 90.63%, respectively). The F-CaCl<sub>2</sub> content for the soil from common radish was reversed, the highest increase for [TMA][PF<sub>6</sub>] occurred at the dosage of 700 mg/kg DM (75.38%) and for [TBA][PF<sub>6</sub>] and [THA][PF<sub>6</sub>] it occurred at 400 mg/kg DM (90.91% and 95.92% respectively).

**Table 2.** Content of fluoride extracted with 0.01 M CaCl<sub>2</sub> (water-soluble) from soil containing one of three quaternary ammonium salts (QAS) (tetramethylammonium hexafluorophosphate [TMA][PF<sub>6</sub>], tetrabutylammonium hexafluorophosphate [TBA][PF<sub>6</sub>], and tetrahexylammonium hexafluorophosphate [THA][PF<sub>6</sub>]) and ammonium hexafluorophosphate [A][PF<sub>6</sub>]. (LSD=least significant difference)

Plant species	Dosage of QAS and [A][PF <sub>6</sub> ] applied to the soil (mg/kg dry matter)	Content of fluoride extracted with 0.01 M CaCl <sub>2</sub> (water-soluble) from soil containing QAS and ammonium salt with the hexafluorophosphate anion (mg F/kg dry matter)			
		[A][PF <sub>6</sub> ]	[TMA][PF <sub>6</sub> ]	[TBA][PF <sub>6</sub> ]	[THA][PF <sub>6</sub> ]
Spring barley	0	1.16 ± 0.19	1.23 ± 0.07	0.99 ± 0.03	0.96 ± 0.02
	1	1.15 ± 0.08	1.27 ± 0.04	1.01 ± 0.02	1.01 ± 0.02
	10	1.22 ± 0.01	1.55 ± 0.02	1.55 ± 0.01	1.19 ± 0.04
	50	1.13 ± 0.01	2.06 ± 0.03	1.59 ± 0.01	1.46 ± 0.01
	100	1.00 ± 0.03	2.07 ± 0.06	1.97 ± 0.05	1.60 ± 0.03
	400	0.96 ± 0.01	2.14 ± 0.03	2.08 ± 0.01	1.69 ± 0.03
	700	0.96 ± 0.02	2.05 ± 0.03	2.20 ± 0.02	1.83 ± 0.06
	1000	1.25 ± 0.02	2.06 ± 0.01	2.09 ± 0.03	1.73 ± 0.05
	LSD (p<0.05)	0.31	0.08	0.11	0.11
Common radish	0	1.06 ± 0.06	1.30 ± 0.01	0.99 ± 0.01	0.98 ± 0.02
	1	1.03 ± 0.06	1.30 ± 0.04	1.03 ± 0.03	1.02 ± 0.02
	10	1.03 ± 0.04	1.41 ± 0.03	1.05 ± 0.06	1.07 ± 0.01
	50	1.05 ± 0.01	1.93 ± 0.04	1.18 ± 0.02	1.05 ± 0.01
	100	0.97 ± 0.01	2.18 ± 0.07	1.50 ± 0.04	1.46 ± 0.01
	400	0.94 ± 0.06	2.14 ± 0.04	1.89 ± 0.01	1.92 ± 0.11
	700	0.98 ± 0.02	2.28 ± 0.08	1.85 ± 0.02	1.86 ± 0.01
	1000	1.14 ± 0.05	2.15 ± 0.01	1.81 ± 0.04	1.83 ± 0.02
	LSD (p<0.05)	0.21	0.10	0.08	0.07

However, it should be emphasized, that in comparison to the amount of fluoride added to the soil with  $[\text{PF}_6]^-$ , the F- $\text{CaCl}_2$  content was low. This is due to a situation in which the  $[\text{PF}_6]^-$  anion in the environment is subjected to hydrolysis to a lesser extent than other anions containing fluoride atoms, e.g., tetrafluoroborate  $[\text{BF}_4]^-$  or hexafluoroantimonate  $[\text{SbF}_6]^-$  <sup>22</sup>. However, Swatłowski et al. <sup>23</sup> suggest that the application of the  $[\text{PF}_6]^-$  anion to the environment may result in the formation of toxic HF.

The content of fluoride extracted with 2 M  $\text{HClO}_4$  (F- $\text{HClO}_4$ ) in the control soil ranged from 2.04 to 2.65 mg/kg dry matter (DM) (Table 3).

**Table 3.** Content of fluoride extracted with 2 M  $\text{HClO}_4$  (potentially available to plants) in soil containing one of three quaternary ammonium salts (QAS) (tetramethylammonium hexafluorophosphate  $[\text{TMA}][\text{PF}_6]$ , tetrabutylammonium hexafluorophosphate  $[\text{TBA}][\text{PF}_6]$ , and tetrahexylammonium hexafluorophosphate  $[\text{THA}][\text{PF}_6]$ ) and ammonium hexafluorophosphate  $[\text{A}][\text{PF}_6]$  with the hexafluorophosphate anion. (LSD=least significant difference)

Plant species	Dosage of QAS and $[\text{A}][\text{PF}_6]$ applied to the soil (mg/kg dry matter)	Content of fluoride extracted with 2 M $\text{HClO}_4$ (potentially available to plants) from soil containing QAS and ammonium salt with the hexafluorophosphate anion (mg F/kg dry matter)			
		$[\text{A}][\text{PF}_6]$	$[\text{TMA}][\text{PF}_6]$	$[\text{TBA}][\text{PF}_6]$	$[\text{THA}][\text{PF}_6]$
Spring barley	0	2.39 ± 0.35	2.04 ± 0.54	2.65 ± 0.09	2.59 ± 0.08
	1	2.51 ± 0.78	2.38 ± 0.18	3.03 ± 0.05	2.73 ± 0.22
	10	3.77 ± 0.17	3.14 ± 0.75	3.70 ± 0.10	3.41 ± 0.04
	50	4.59 ± 0.07	4.47 ± 0.52	4.68 ± 0.13	4.77 ± 0.12
	100	6.32 ± 0.14	6.01 ± 0.48	8.07 ± 0.15	8.57 ± 0.09
	400	18.69 ± 0.48	12.78 ± 0.33	16.06 ± 0.24	21.05 ± 0.81
	700	28.56 ± 2.25	16.65 ± 0.99	24.16 ± 0.43	33.51 ± 0.34
	1000	42.15 ± 1.54	32.41 ± 1.44	35.17 ± 0.73	45.41 ± 0.65
	LSD (p<0.05)	1.88	2.34	2.15	1.77
Common radish	0	2.47 ± 0.01	2.28 ± 0.07	2.52 ± 0.06	2.53 ± 0.07
	1	2.56 ± 0.13	2.67 ± 0.07	2.97 ± 0.17	2.62 ± 0.02
	10	2.79 ± 0.12	2.85 ± 0.39	3.47 ± 0.19	3.53 ± 0.05
	50	3.95 ± 0.21	4.39 ± 0.03	4.27 ± 0.02	4.38 ± 0.17
	100	6.72 ± 0.61	5.90 ± 0.02	6.52 ± 0.11	6.94 ± 0.09
	400	14.72 ± 1.10	17.36 ± 1.76	16.53 ± 0.25	18.88 ± 0.58
	700	28.73 ± 1.50	28.67 ± 0.75	27.41 ± 0.67	34.20 ± 0.75
	1000	41.38 ± 0.32	36.00 ± 0.08	36.55 ± 1.91	44.84 ± 0.72
	LSD (p<0.05)	2.49	1.75	2.61	2.18

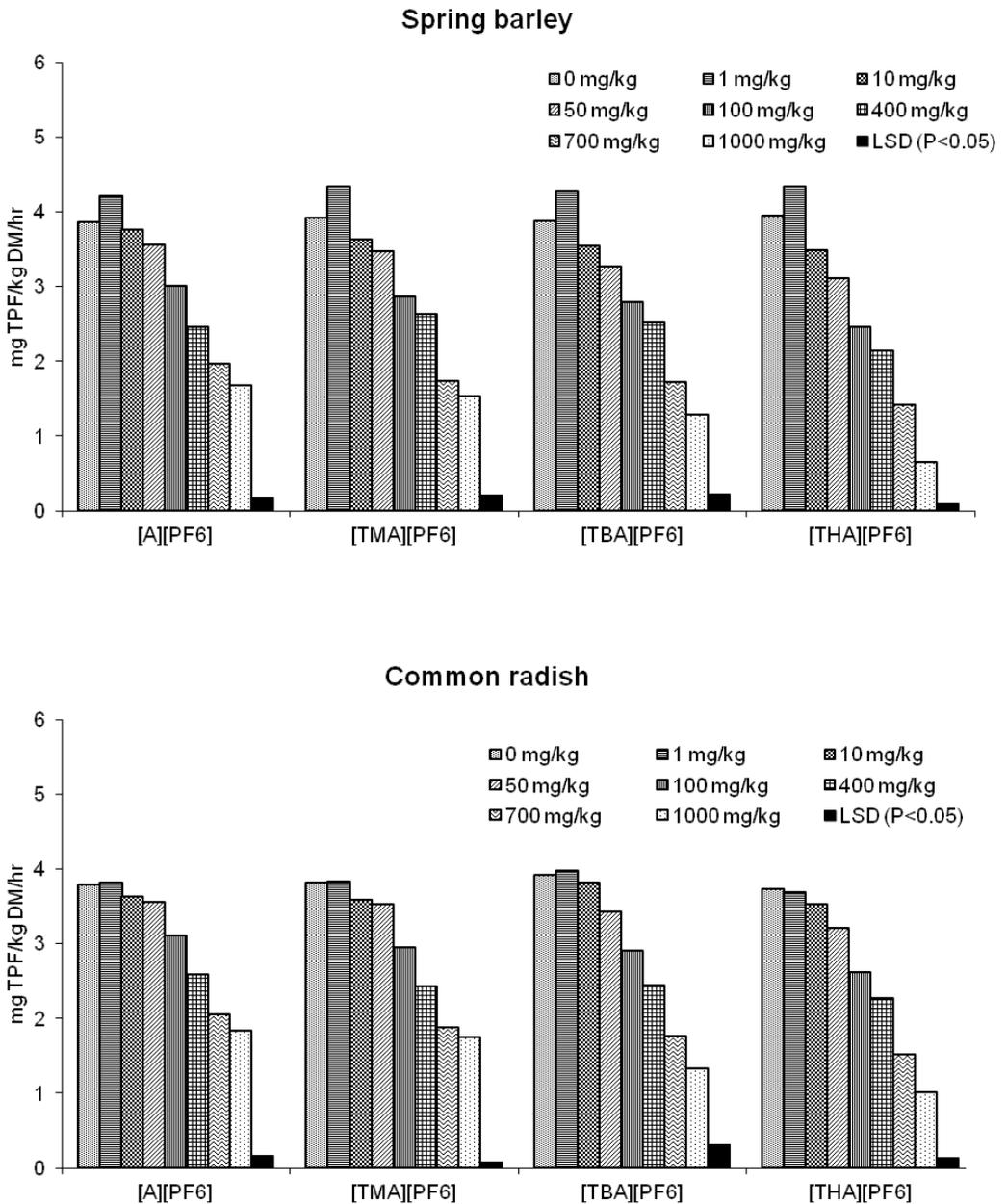
The addition of the inorganic salt  $[\text{A}][\text{PF}_6]$ , as well as all the QAS resulted in the increase of F- $\text{HClO}_4$  content, both in the soil from spring barley and in the soil from

common radish, increasing with the increment of dosage of the analyzed compounds. However, for [A][PF<sub>6</sub>], [TMA][PF<sub>6</sub>], and [TBA][PF<sub>6</sub>], the observed changes for dosages up to 50 mg/kg DM were not statistically significant. In the case of [THA][PF<sub>6</sub>] no statistically significant influence was observed only for dosages higher than 10 mg/kg DM. The highest increase of F-HClO<sub>4</sub> was determined after the addition of the highest dosage of all the analyzed compounds. Compared to the control for [A][PF<sub>6</sub>], [TMA][PF<sub>6</sub>], [TBA][PF<sub>6</sub>], and [THA][PF<sub>6</sub>] it was 1663.60%, 1488.72%, 1227.17%, and 1653.28% in the soil from spring barley, respectively, and in the soil from common radish it was 1575.30%, 1478.95%, 1350.40%, and 1672.33%, respectively. A comparison of QAS clearly demonstrates that the F-HClO<sub>4</sub> content increased to the greatest extent after the application of [THA][PF<sub>6</sub>]<sub>2</sub> which is a quaternary ammonium salt with the longest alkyl substituents. Freire et al.<sup>5</sup> showed that the level of hydrolysis of [BF<sub>4</sub>]<sup>-</sup> and [PF<sub>6</sub>]<sup>-</sup> anions rises with an increasing length of alkyl substituents in cations. Moreover, many researchers believe that it is the presence of fluoride in the anions of ionic liquids that is largely responsible for the ecotoxicity of these substances.<sup>22-26</sup>

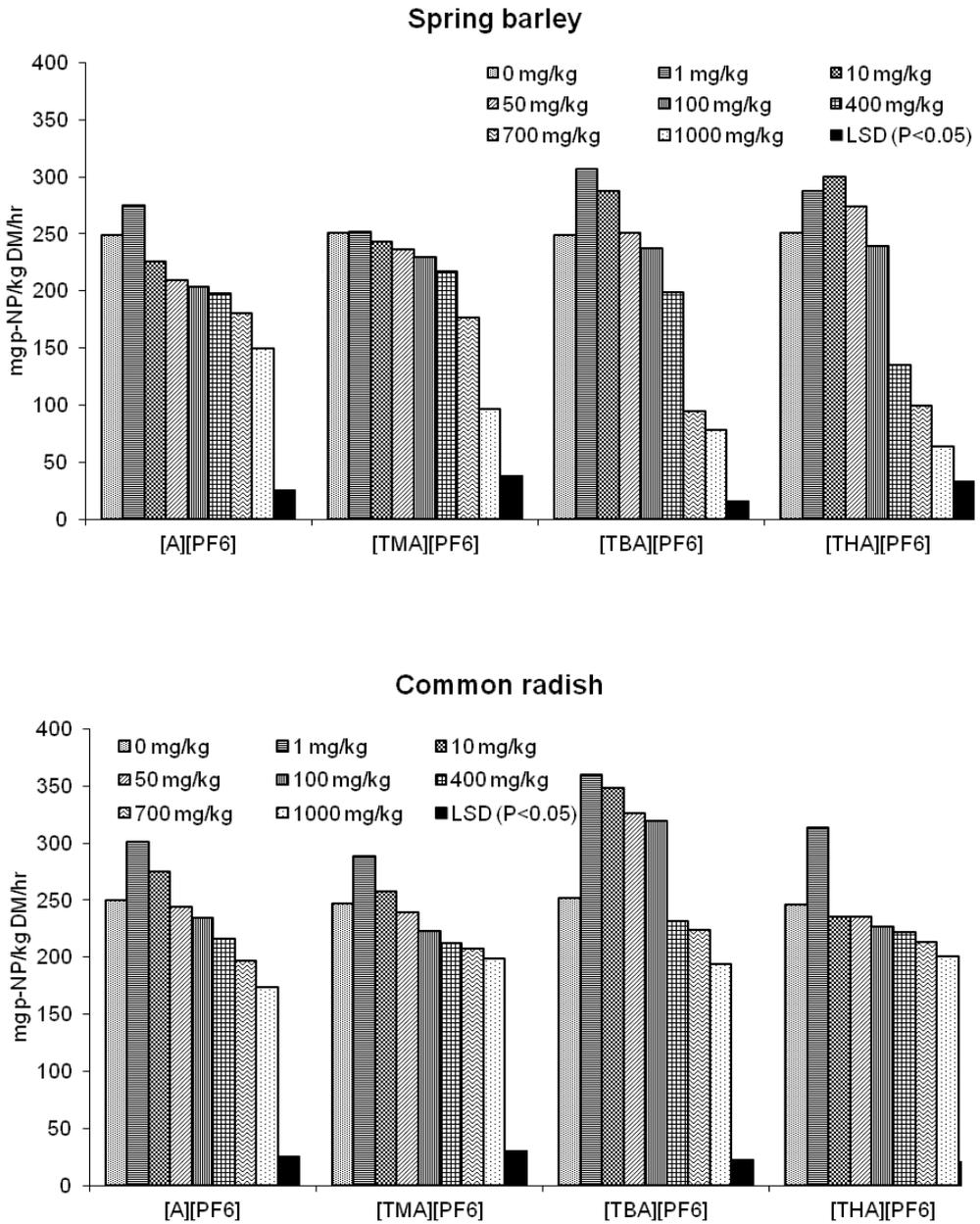
One of the good markers of changes occurring in the soil environment under the influence of natural and anthropogenic factors is the activity of enzymes causing transformation of its constituents.<sup>27</sup> Changes in soil enzymatic activities are among the earliest signals indicating changes in the intensity of environmental life processes. This phenomenon results from the situation where many chemical compounds assume a toxic or mutagenic character after the metabolic transformations occurring in living organisms.<sup>28</sup>

The application of QAS and [A][PF<sub>6</sub>] to soil at dosages from 1 to 50 mg/kg DM usually had no significant effect or increased activity of the determined soil enzymes. In comparison to control, the highest activity stimulation in soil from both test plants was recorded for acid phosphatase after the application of [TBA][PF<sub>6</sub>] at the dosage of 1 mg/kg DM (spring barley: 23.55% and common radish: 43.08%). In the case of the dehydrogenases, dosages of 10 mg/kg DM and 50 mg/kg DM for all the analyzed substances caused a statistically significant drop in the activity (Figure 1).

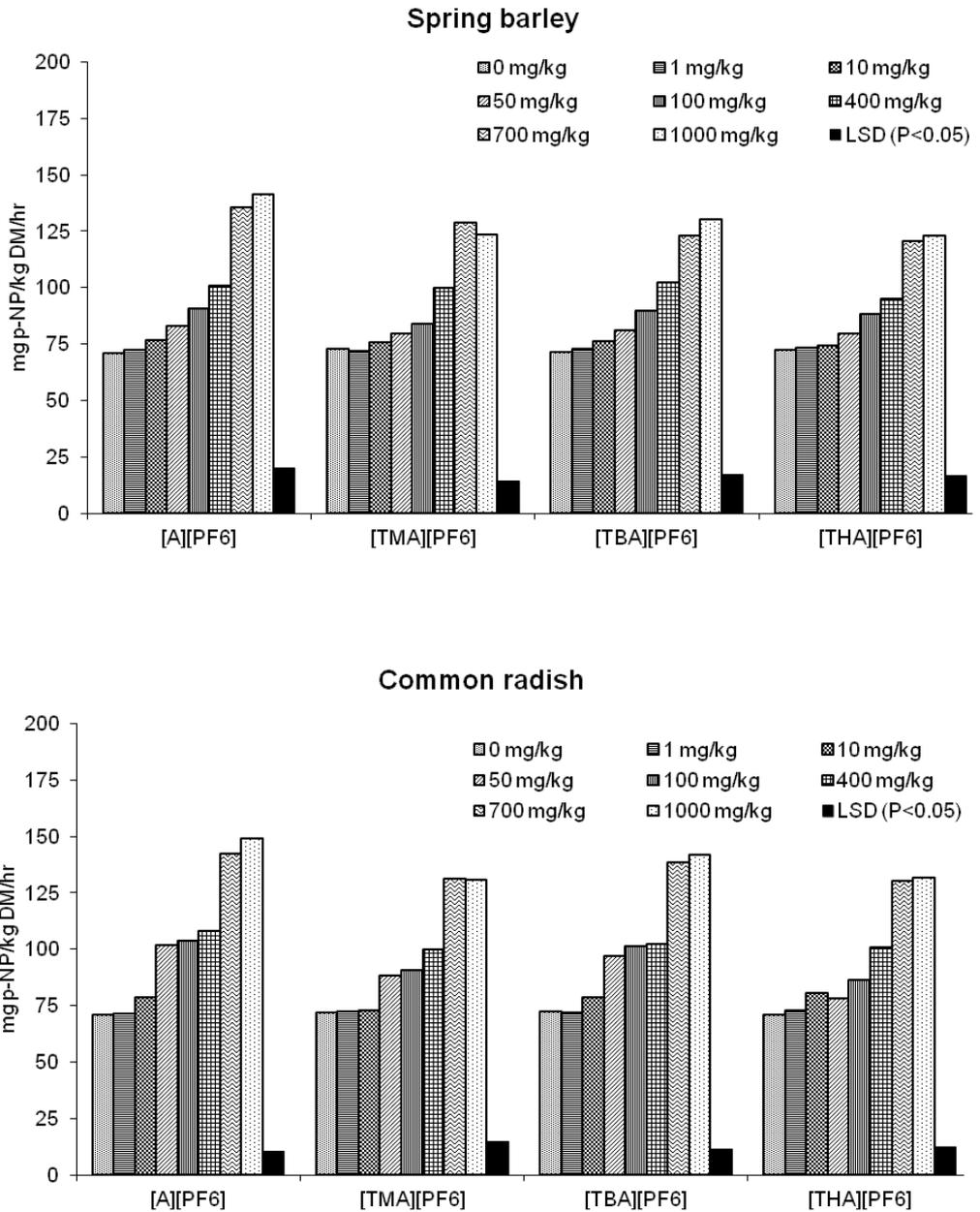
The application of the dosage of 100 mg/kg DM had a variable effect on the activity of the determined enzymes, depending on the type of compound as well as the tested plant species. At this dosage, the activity of dehydrogenases in soil from both test plants containing QAS and [A][PF<sub>6</sub>] went through a statistically significant decrease compared to control. Moreover, the observed effect increased with the extension of the alkyl chains in the cation and for [THA][PF<sub>6</sub>] it was 37.56% in the soil from spring barley and 29.76% in the soil from common radish. A statistically significant inhibition of acid phosphatase activity occurred only after the application of [A][PF<sub>6</sub>] to the soil from spring barley at 15.72% (Figure 2). However, in the case of alkaline phosphatase the 100 mg/kg DM dosage usually resulted in statistically significant stimulation of this enzyme's activity, achieving 28.34% and 22.53% for [A][PF<sub>6</sub>], for the soil from spring barley and common radish, respectively (Figure 3). On the other hand, *o*-diphenol oxidase activity in the soil from spring barley was not subjected to significant changes, whereas a drop in activity was observed for the soil from common radish, which for [A][PF<sub>6</sub>], [TMA][PF<sub>6</sub>], and [TBA][PF<sub>6</sub>] was 8.78%, 14.87%, and 7.71%, respectively (Figure 4).



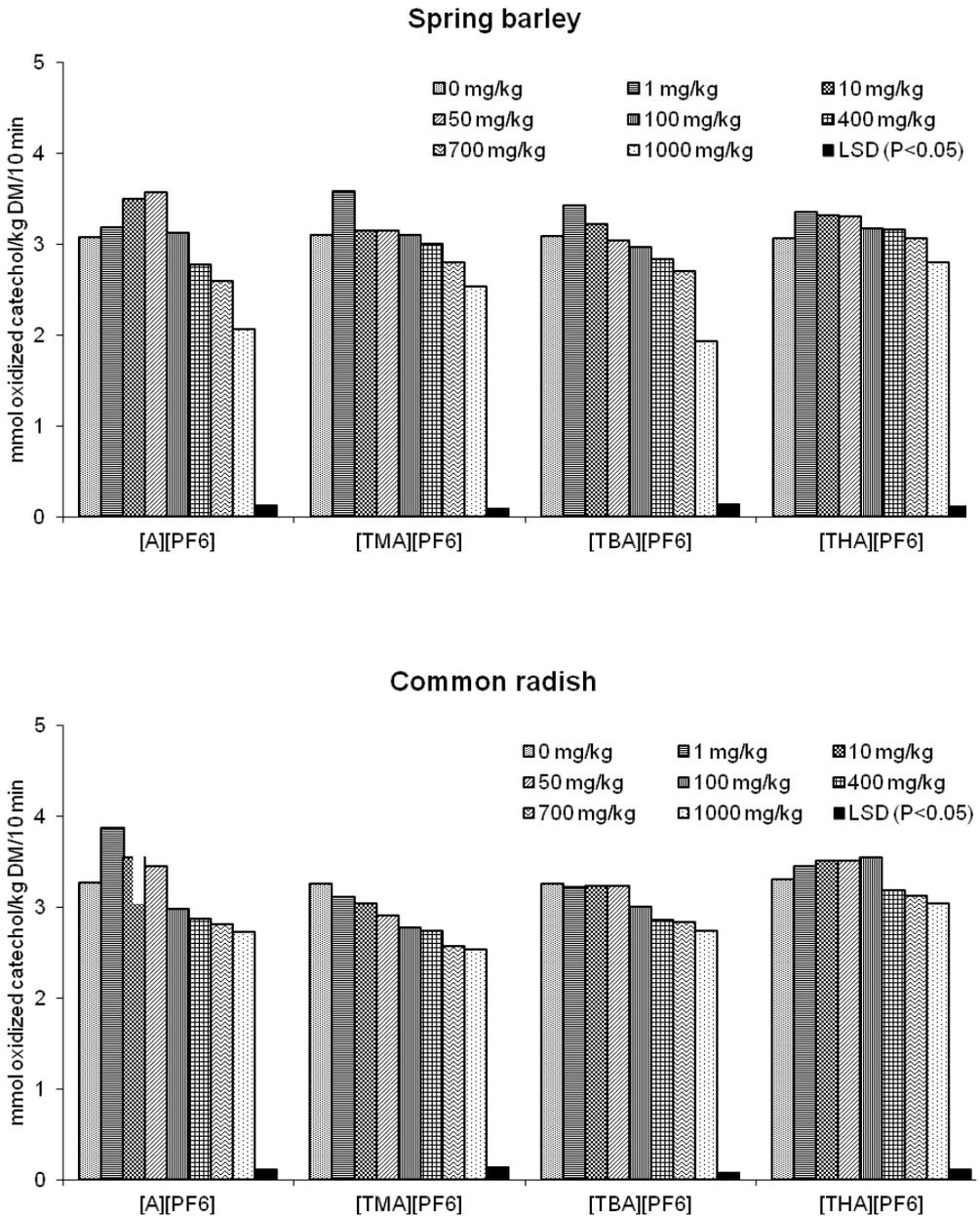
**Figure 1.** Activity of dehydrogenases, mg triphenylformazan (TPF)/kg dry matter/hour, in soil containing 0, 1, 10, 50, 100, 400, 700 and 1000 mg/kg dry matter, of the ammonium hexafluorophosphate [A][PF6], and quaternary ammonium salts (QAS): tetramethylammonium hexafluorophosphate [TMA][PF6], tetrabutylammonium hexafluorophosphate [TBA][PF6], and tetrahexylammonium hexafluorophosphate [THA][PF6].



**Figure 2.** Activity of acid phosphatase, mg p-nitrophenol (p-NP)/kg dry matter/hour, in soil containing 0, 1, 10, 50, 100, 400, 700 and 1000 mg/kg dry matter, of the ammonium hexafluorophosphate [A][PF6], and quaternary ammonium salts (QAS): tetramethylammonium hexafluorophosphate [TMA][PF6], tetrabutylammonium hexafluorophosphate [TBA][PF6], and tetrahexylammonium hexafluorophosphate [THA][PF6].



**Figure 3.** Activity of alkaline phosphatase, mg p-nitrophenol (p-NP)/kg dry matter/hour, in soil containing 0, 1, 10, 50, 100, 400, 700 and 1000 mg/kg dry matter, of the ammonium hexafluorophosphate [A][PF6], and quaternary ammonium salts (QAS): tetramethylammonium hexafluorophosphate [TMA][PF6], tetrabutylammonium hexafluorophosphate [TBA][PF6], and tetrahexylammonium hexafluorophosphate [THA][PF6].



**Figure 4.** Activity of o-diphenol oxidase, mmol oxidized catechol/kg dry matter/10 minutes, in soil containing 0, 1, 10, 50, 100, 400, 700 and 1000 mg/kg dry matter, of the ammonium hexafluorophosphate [A][PF6], and quaternary ammonium salts (QAS): tetramethylammonium hexafluorophosphate [TMA][PF6], tetrabutylammonium hexafluorophosphate [TBA][PF6], and tetrahexylammonium hexafluorophosphate [THA][PF6].

Application of all analyzed compounds at dosages from 400 mg/kg DM to 1000 mg/kg DM primarily caused significant changes of soil enzyme activities. Only in the soil from spring barley, we observed no significant effect of [TMA][PF<sub>6</sub>] at the dosage of 400 mg/kg DM on the activity of acid phosphatase and [THA][PF<sub>6</sub>] at 400 mg/kg DM and 700 mg/kg DM dosages on the activity of *o*-diphenol oxidase. On the other hand, the addition of [THA][PF<sub>6</sub>] at the dosages of 400 mg/kg DM and 700 mg/kg DM did not have a significant effect on the activity of acid phosphatase or on the activity of *o*-diphenol oxidase. In the remaining cases, a statistically significant inhibition of dehydrogenases, acid phosphatase, and *o*-diphenol oxidase activity and stimulation of alkaline phosphatase activity occurred, increasing with the increment of dosage of the analyzed compounds. In the soil from spring barley, the greatest drop of dehydrogenases and acid phosphatase activity was observed after the addition of [THA][PF<sub>6</sub>] at the dosage of 1000 mg/kg DM (83.50% and 74.66%, respectively), and the activity of *o*-diphenol oxidase after the application of [TBA][PF<sub>6</sub>] at the dosage of 1000 mg/kg DM (37.20%). On the other hand, in the soil from common radish, the demonstrated effect for dehydrogenases, acid phosphatase, and *o*-diphenol oxidase was observed after application of [THA][PF<sub>6</sub>] at the dosage of 1000 mg/kg DM (72.92%), [TBA][PF<sub>6</sub>] at 1000 mg/kg DM (22.91%), and [TMA][PF<sub>6</sub>] at 1000 mg/kg DM (22.13%). The activity of alkaline phosphatase in the soil from both test plants was stimulated to the greatest extent after the application of the inorganic salt [A][PF<sub>6</sub>] at the dosage 1000 mg/kg DM (100.16% for spring barley and 110.18% for common radish).

The literature provides only a few reports on the effect of ionic liquids on the enzymatic activity of soils. However, these publications show primarily a negative effect of the substances on soil enzymes.<sup>29,30</sup> Furthermore, many authors have determined the antimicrobial activity of ionic liquids.<sup>31–34</sup> Moreover, Pernak et al.<sup>35</sup> state that the antimicrobial activity of ionic liquids indicates a negative influence of these compounds on the environment, and also biodegradation difficulties. Stasiewicz et al.<sup>36</sup> observed that the alkyl substituent extension in cations accelerates the biodegradation process, and simultaneously increase the toxicity of these substances. This is corroborated by the results of the present study, which show that as the number of carbon atoms in alkyl chains increases, there is also an increase in the effect of QAS and ammonium hexafluorophosphate on dehydrogenases, phosphatase, and *o*-diphenol oxidase. This may also stem from the greater affinity of a longer alkyl substituent for the hydrophobic surfaces of soil particles.<sup>37</sup>

As was mentioned above, the presence of fluoride atoms in anions is largely responsible for the ecotoxicity of ionic liquids.<sup>22–26</sup> The calculated Pearson's correlation coefficients showed a significant ( $p < 0.05$ ) negative correlation between the content of both of the forms of fluoride (F-CaCl<sub>2</sub> and F-HClO<sub>4</sub>) and the activity of dehydrogenases, acid phosphatase, and *o*-diphenol oxidase (Table 4). On the other hand, the activity of alkaline phosphatase was positively correlated to the fluoride content in soil. It should be emphasized, that the values of correlation coefficients were higher for F-HClO<sub>4</sub> than for F-CaCl<sub>2</sub>. The negative effect of fluoride on the enzymatic activity of soils has been determined by many researchers.<sup>38–41</sup> Furthermore, Telesiński et al.<sup>42</sup> reported a significant negative correlation between

the fluoride content and the value of the adenylate energy charge (AEC), which indicates the physiological state of soil microorganisms.

**Table 4.** Pearson's correlation coefficients between the fluoride content and enzyme activities in soil containing one of three quaternary ammonium salts (QAS) (tetramethylammonium hexafluorophosphate, tetrabutylammonium hexafluorophosphate, and tetrahexylammonium hexafluorophosphate) and ammonium hexafluorophosphate with the hexafluorophosphate anion. (DHA=dehydrogenase activity;  $P_{ac}$ =acid phosphatase;  $P_{al}$ =alkaline phosphatase; o-DPO=o-diphenol oxidase activity; F-CaCl<sub>2</sub>=water soluble fluoride extracted with 0.01M CaCl<sub>2</sub>; F-HClO<sub>4</sub>=fluoride potentially available to the plant extracted with 2M HClO<sub>4</sub>)

Fluoride form	Soil enzyme activity			
	DHA	$P_{ac}$	$P_{al}$	o-DPO
F-CaCl <sub>2</sub>	-0.59*	-0.47*	0.43*	-0.49*
F-HClO <sub>4</sub>	-0.93*	-0.75*	0.92*	-0.70*

\*significant at  $p < 0.05$

## CONCLUSIONS

The application of QAS and ammonium hexafluorophosphate containing the hexafluorophosphate anion to soil caused the fluoride content to increase, particularly in the form that was potentially available to plants. The changes in the fluoride content of soil were negatively correlated to the activity of soil enzymes, such as dehydrogenases, acid phosphatase, and o-diphenol oxidase and positively correlated to alkaline phosphatase activity. The dehydrogenase activity showed the greatest changes. Moreover, the observed effect increased with the increment of the dosage of the analyzed compounds and increased multiple times as the number of carbon atoms in alkyl substituents in the cations increased.

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