© by PSP Volume 31– No. 07/2022 pages 7140-7147 Fresenius Environmental Bulletin

EFFECT OF COMBINED POLLUTION OF LEAD AND BENZO[A]PYRENE ON ABSORPTION OF LEAD BENZO[A]PYRENE AND MINERAL ELEMENTS IN RYEGRASS

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ABSTRACT

The lead (Pb) and benzo[a]pyrene (B[a]P) absorption and transfer characteristics of ryegrass under the Pb-B[a]P combined pollution and their influences on mineral absorption were investigated through a laboratory pot-culture experiment. Results demonstrate that Pb-B[a]P combined pollution increase Pb and B[a]P contents in ryegrass significantly. Low contaminated concentration of Pb (<100mg·kg^{-1}) and B[a]P are easy to accumulate in root system of ryegrass, while only few Pb and B[a]P migrate to shoots. When the Pb contaminated concentration is 100 mg·kg^{-1} and B[a]P contaminated concentration is 30 mg·kg^{-1}, the bioconcentration factor (BCF) and transfer coefficient (TC) of both shoots and roots of ryegrass are higher than those under other concentrations, indicating that ryegrass achieves stronger remediation effect to low-concentration Pb (100mg·kg^{-1}) and B[a]P (30mg·kg^{-1}) combined pollution. Pb-B[a]P combined stress hinders mineral absorption of ryegrass. With the increase of Pb and B[a]P contaminated concentrations, N, P, K, Mg and Fe contents in ryegrass are decreased dramatically. The root system of ryegrass is the main absorption and bioconcentration organ of N, P, K and Fe. Pb-B[a]P combined pollution is negatively correlated with N, K, Mg and Fe contents in roots and shoots.

KEYWORDS:
Pb-B[a]P combined stress, mineral elements, absorption and accumulation, multiple regression analysis, ryegrass

INTRODUCTION

As two types of typical soil pollutants, heavy metals and polycyclic aromatic hydrocarbons (PAHs) usually co-exist [1]. At present, relevant researches on ecological effects of recombination and co-existence between heavy metals and PAHs have obtained certain research achievements while researches on ecological effects of combined heavy metals-PAHs pollution are still quite insufficient [2,3,4]. As typical representatives of heavy metals and PAHs, lead (Pb) and benzo[a]pyrene(B[a]P) have enormous environmental hazards and relatively enduring pollution, so they have always been research hotspots, but there are few researches on combined pollution of the two and there is a lack of consideration of long-term exposure of common pollutants of medium and low contents in practice [5].

Currently, physical, chemical and biological remediation techniques are mainly adopted to recover soils polluted by heavy metals and polycyclic aromatic hydrocarbons. As one of bioremediation techniques, phytoremediation is to absorb pollutants in the environment by plants and reduces heavy metal contents in soil by plant harvest. It is an emerging, high-efficient ecological-friendly in-situ remediation technique [6,7].

Phytoremediation technology is widely applied to remediation of environment polluted by heavy metals and polycyclic aromatic hydrocarbon as a result of its advantages of high efficiency, low cost, environmental-friendly and easy implementation in heavy metals absorption and transfer as well as PAHs absorption and desorption. Dushenkov et al. discovered that immersion of multiple hydroponic terrestrial plants into heavy metal solution could eliminate heavy metals in water effectively [8].

Ryegrass is a plant which is planted extensively, grows quickly and has high biomass. The strong root system and root exudates are appropriate for absorption and accumulation of heavy metals as well as degradation and mineralization of PAHs [9]. Gao et al. proved that ryegrass could accelerate PAHs removal in water and shortened the half-time period of pyrene removal by 47.4% compared with that of the control group [10]. Therefore, ryegrass has profound research values in remediation function, mechanism and regulation technologies of heavy metal polluted soils. However, there are few studies on plant remediation of heavy metals-PAHs combined pollution, especially to studies on Pb-B[a]P combined pollution.

In this study, Pb and B[a]P accumulation characteristics of different parts of ryegrass were investigated under Pb-B[a]P combined pollution with different mixing ratios. Moreover, influences of combined pollutants on mineral absorption and transfer of ryegrass were analyzed and the potential ryegrass in remediation of Pb-B[a]P combined polluted soils was explored, aiming to provide some scientific references for further studying regulation technologies of ryegrass in safe remediation of Pb-B[a]P polluted soils.

MATERIALS AND METHODS

Experimental materials. All reagents used in this study are of analytic grade. Seeds of ryegrass were commercially available from Liaoning Fuyou seeds Co. Ltd. in Shenyang. Pb and B[a]P were purchased from Sigma-Aldrich. Test solutions of B[a]P were prepared by dissolving in 0.1% acetone and making up with deionized water while $Pb(NO_3)_2$ was prepared with deionized water only. Control treatments were also exposed to 0.1% acetone. B[a]P and exogenous Pb were sprayed and applied into soil respectively.

Experimental design. According to the concentrations of Pb and B[a]P used in studies on the effect of Pb and B[a]P on plants [11], and taking into the current pollution situation of Pb and B[a]P in the Liaoning Province, the development trend of Pb and B[a]P in China, as well as possible accidental leaking of Pb and B[a]P into the environment, four contaminated concentrations of Pb (0, 100, 200, 400 $mg·kg^{-1}$) and five contaminated concentrations of B[a]P (0, 10, 30, 50, 100 $mg·kg^{-1}$) were selected for the pot experiment. Altogether, twenty different treatments were applied to the soil, and each treatment had three replications (Table 1).

The tested soil was black soil, with the following soil parameters: soil pH 6.23, organic matter 20.17 $g·kg^{-1}$, total N and P 3.64 and 3.89 $g·kg^{-1}$, respectively, cation exchange capacity 20.34 $cmol·kg^{-1}$. Air dried unpolluted soil was sieved through a 4-mm sieve and thoroughly mixed with basal fertilizers, and then, spiked with Pb and B[a]P at levels as shown in Table 1. After five weeks incubation mixed, the soil was placed into plastic pots (30 cm in height, 40 cm in diameter), and ready for the pot-culture experiment.

The pot-culture experiment was conducted in the Ecological Experimental Garden of Liaoning University. Plump ryegrass seeds were selected, treated with 60% (v/v) ethanol for 15 min for surface sterilization, followed with repeated washing with distilled water, and then soaked in thermostat for 24h at 25±2°C. Then the seeds were sown in the individual plastic pots, which were placed under controlled temperature of 25±2°C, 60-65% relative humidity and natural daylight. Pots were carefully irrigated with water as necessary.

After 70 days of treatment, the ryegrass were washed with distilled water and dried prior to record the sample weight at harvest. Plant parts (shoots and roots) were dried at 90°C for 72 h until constant weight.

Analysis. A portion of dried shoot and root samples were filtered through a 2 mm nylon screen. Soxhlet extraction was performed in accordance with the USA EPA Standard Method, in which 5 g of plant sample was added to 10 g of anhydrous sodium sulphate (S6264, Sigma Chemical Co.). The mixture was transferred into a cellulose extraction thimble and inserted into a Soxhlet fitted with a 250 mL flask. A dichloromethane-acetone solution (150 mL, v:v 1:1) was added, and the whole set up was heated for 18 h in a water bath at 69 °C. The extracts were concentrated to 10 mL by a rotary evaporator and used for subsequent clean up. Clean-up steps were performed before analysis to remove the organic and inorganic constituents other than those of interest. Florisil column clean-up was thus applied to purify the concentrated extract. The extracts were concentrated to less than 5 mL by a rotary evaporator, and then n-hexane (10 mL) was added and concentrated to less than 2 mL. Subsequently, B[a]P was injected into the sample extract as internal standards for quantitation. Finally, the extract was topped up to 2 mL with n-hexane for the analysis of B[a]P. GC-MS analysis was carried out on a Hewlett Packard 6890 GC system equipped with a mass selective detector and a 30 m×0.25 mm×0.25 m DB-5 capillary column (J & W Scientific Co., Ltd., USA).

A portion of dried shoot and root samples were ground into fine powders (2 mm mesh). After milling and acid digestion of 0.5 g dried shoot and root samples with H_2SO_4-H_2O_2, the N concentrations of all shoots and roots were determined by the Kjeldahl digestion method, the P concentrations by Mo-Sb colorimetric method, and the Pb, K, Mg, Fe concentrations by TAS-990 atomic absorption spectrophotometer [12].

All reagents were supra-pure and high-purity water was employed throughout. A certified reference material (GBW07605, from the National Research Center for Standard Materials in China) was used to monitor the recovery of B[a]P and elements from the plant samples. The analytical results showed no signs of contamination and that the precision and bias of the analysis were generally <10% for B[a]P and elements. The recovery rate for the certified reference material was 91-107%. Data were expressed on a dry weight basis.

Bioconcentration factor (BCF) was calculated as the ratio between the Pb or B[a]P concentration in the roots and that in the soils. Root-to-shoot transfer coefficient (TC) indicating the mobility of Pb or B[a]P from roots to shoots was also computed as the ratio between the Pb or B[a]P concentration in shoots and roots [13].

TABLE 1
Experimental design of different treatments (mg·kg^{-1})

Treatment	Pb	B[a]P
T1(Control)	0	0
T2	0	10
T3	0	30
T4	0	50
T5	0	100
T6	100	0
T7	100	10
T8	100	30
T9	100	50
T10	100	100
T11	200	0
T12	200	10
T13	200	30
T14	200	50
T15	200	100
T16	400	0
T17	400	10
T18	400	30
T19	400	50
T20	400	100

TABLE 2
The variations of Pb and B[a]P in various parts of ryegrass(mg·kg^{-1})

Treatment	Pb			B[a]P		
	Soils	Shoots	Roots	Soils	Shoots	Roots
T1	0.02a	0.00a	0.00a	0.01a	0.00a	0.00a
T2	0.03a	0.00a	0.00a	0.34a	0.37a	0.45a
T3	0.05a	0.00a	0.00a	10.44b	2.47b	6.51b
T4	0.04a	0.00a	0.00a	24.47c	6.48c	13.58c
T5	0.02a	0.00a	0.00a	60.26d	20.01d	31.21d
T6	95.22b	2.79b	15.42b	0.01a	0.00a	0.00a
T7	98.16b	2.99b	16.98b	0.38a	0.27a	0.45a
T8	101.92b	3.06b	17.98b	12.81b	4.73b	8.35b
T9	104.44b	3.14b	18.84b	25.50c	8.04c	15.70c
T10	102.97b	2.98b	19.92b	66.68d	27.38d	38.05d
T11	154.45c	7.05c	32.23c	0.01a	0.00a	0.00a
T12	168.28c	10.14c	34.96c	0.50a	0.29a	0.47a
T13	172.31c	18.50c	42.65c	13.27b	5.53b	9.94b
T14	187.78c	23.41c	45.76c	27.27c	9.53c	16.94c
T15	193.52c	29.84c	58.25c	71.03d	32.31d	43.08d
T16	317.39d	67.95d	108.93d	0.01a	0.00a	0.00a
T17	322.25d	69.51d	113.93d	0.60a	0.34a	0.71a
T18	334.24d	75.38d	118.93d	15.86b	7.03b	10.56b
T19	342.16d	79.40d	133.45d	33.64c	11.43c	18.76c
T20	351.76d	82.91d	140.41d	79.55e	43.85e	53.86e

Notes: Different lowercase letters in the Table represent that significant differences at $p < 0.01$ level between different Pb and B[a]P treatments.

Data analysis. The values were averaged from three replicates and the error bars corresponded to standard error of the mean. A one-way analysis of variance (one-way ANOVA) followed by least significant difference (LSD) test was used to determine the statistical significance ($p < 0.05$) of each parameter among treatments performed on SPSS version 23.0. The detailed regression analysis by Origin 8.5 is provided in the SI.

RESULTS AND DISCUSSION

The migration and accumulation characteristics of Pb and B[a]P in soil-ryegrass system. It can be seen from Table 2 that compared with the contrast, Pb or B[a]P contents in shoots and roots of ryegrass are increased significantly, which are attributed to the increase of Pb or B[a]P contents in soils. According to correlation analysis, Pb or B[a]P contents in ryegrass plant are significantly correlated with Pb or B[a]P contents in soils (p<0.01). Under Pb-B[a]P combined pollution conditions, Pb or B[a]P contents in shoots of ryegrass are lower than those in roots. The B[a]P contents in shoots are 17.78%-62.06% lower than those in roots. Influences on Pb contents are even more serious. When Pb contaminated concentration is <100 mg·kg^{-1}, Pb contents in shoots are 81.91%-99.52% lower than those in roots. When Pb contaminated concentration is 400 mg·kg^{-1}, Pb contents are accumulated more in shoots and Pb contents in the shoots are 36.62%-40.95% lower than those in roots. These reflect that low-concentration Pb (<100mg·kg^{-1}) and B[a]P are easier to accumulate in ryegrass root system and only few are transferred to shoots.

Higher bioconcentration factor (BCF) indicates the higher absorption and accumulation capacity of plants to pollutants. Accordingly, it is beneficial for plants to extract and remediate polluted soils [14]. It can be seen from Table 3 that in all treatments, the BCF of Pb in roots of ryegrass was higher than that in shoots. When Pb contaminated concentration are 100 and 200 mg·kg^{-1}, the BCF in shoots and roots are higher than 1. When Pb contaminated concentration is 400 mg·kg^{-1}, the BCF in shoots is lower than 1, whereas the BCF in roots is higher than 1. These demonstrate that when Pb contaminated concentration are 100 and 200 mg·kg^{-1}, ryegrass has strong Pb accumulation ability. With the increase of Pb contents, the Pb accumulation ability of ryegrass decreases.

TABLE 3
The BCF of Pb and B[a]P in ryegrass

Treatment	Shoots		Roots	
	Pb	B[a]P	Pb	B[a]P
T1	0.000a	0.000a	0.002a	0.000a
T2	0.000a	1.236b	0.002a	1.655b
T3	0.000a	2.039c	0.002a	2.318c
T4	0.000a	0.928d	0.002a	1.216d
T5	0.000a	0.723e	0.002a	0.824e
T6	2.019b	0.000a	3.062e	0.000a
T7	2.020b	2.480b	3.073e	2.631b
T8	2.023b	3.191c	3.076e	3.387c
T9	2.025b	2.173d	3.080e	2.357d
T10	2.029b	1.167e	3.093e	1.283e
T11	1.046c	0.000a	2.109b	0.000a
T12	1.050c	1.791b	2.108b	2.949b
T13	1.058c	2.815c	2.148b	3.207c
T14	1.065c	1.854d	2.174b	2.521d
T15	1.074c	1.013e	2.191b	1.240e
T16	0.943d	0.000a	1.813c	0.000a
T17	0.954d	1.335b	1.865c	1.532b
T18	0.961d	2.041c	1.821c	2.371c
T19	0.920d	1.031d	1.856c	1.316d
T20	0.939d	1.027d	1.878c	1.284d

TABLE 4
The TC of Pb and B[a]P in ryegrass

Treatment	Pb	B[a]P
T1	0.003a	0.000a
T2	0.003a	0.922b
T3	0.003a	1.079c
T4	0.003a	0.877d
T5	0.003a	0.641e
T6	0.962d	0.000a
T7	0.959d	0.960b
T8	0.953d	1.212c
T9	0.951d	0.866d
T10	0.946d	0.670e
T11	0.735c	0.000a
T12	0.736c	0.917b
T13	0.733c	1.356c
T14	0.729c	0.863d
T15	0.721c	0.650e
T16	0.519b	0.000a
T17	0.517b	0.979b
T18	0.515b	1.319c
T19	0.513b	0.843d
T20	0.510b	0.616e

In all treatments, the BCF of B[a]P in roots of ryegrass is higher than that in shoots. When B[a]P contaminated concentration is >30 mg·kg^{-1}, the BCF in shoots and roots of ryegrass are higher than 1. When the B[a]P contaminated concentration is 30mg·kg^{-1}, BCF in all parts of ryegrass reach the maximum. Under Pb-B[a]P combined pollution, the BCF of B[a]P is the highest when Pb contaminated concentration is 100 mg·kg^{-1}. This indicates that under 100 mg·kg^{-1} Pb stress, B[a]P has strong accumulation capacity. The bioconcentration ability of B[a]P decreases gradually with the increase of Pb contaminated concentration.

BCF is a physical parameter that reflects heavy metals absorption and accumulation characteristics of plants and it is used to evaluate bioconcentration ability of plants to absorb and transfer heavy metals into the plant body. In this study, BCF of shoots and roots of ryegrass are higher than 1 when Pb contaminated concentrations are 100 and 200 mg·kg^{-1}. BCF of shoots and roots of ryegrass are higher than 1 when B[a]P contaminated concentrations are >30 mg·kg^{-1}. When Pb contaminated concentration is 100 mg·kg^{-1} and B[a]P contaminated concentration is 30 mg·kg^{-1}, BCF of shoots and roots of ryegrass are higher than those in other treatments. When Pb contaminated concentration is 100 mg·kg^{-1}, ryegrass has strong Pb bioconcentration ability. The Pb bioconcentration ability of ryegrass decreases with the increase of the Pb contaminated concentration. Both shoots and roots of ryegrass have strong B[a]P resistance under Pb-B[a]P combined pollutions. The B[a]P bioconcentration ability of ryegrass decreases gradually when Pb contaminated concentrations are higher than 100 mg·kg^{-1}.

Higher transfer coefficient (TC) implies the stronger migration ability of pollutants from root system to shoots [14]. In Table 4, TC of Pb and B[a]P in all treatments are significantly higher than those in the control. The TC of Pb and B[a]P are significantly among different treatments. TC of Pb and B[a]P are positively related with Pb and B[a]P contents in soils (p<0.05). Therefore, ryegrass has relatively good bioconcentration characteristics and high transfer ability. It is suggested to use ryegrass to remediate low-concentration Pb and B[a]P polluted soils.

TC is used to evaluate the ability of plants that transfers heavy metals from the roots to the shoots. Higher TC implies the stronger ability of plants to transfer heavy metals from roots to the shoots. This study concludes that under Pb-B[a]P combined pollution, the TC of Pb (>0.950) in ryegrass are high when the Pb contaminated concentrations (100 mg·kg^{-1}) are low. Under low Pb contaminated concentrations, ryegrass absorbs and transfers Pb from roots to shoots, showing strong remediation potential to low-concentration Pb polluted soils. When B[a]P contaminated concentration is 30 mg·kg^{-1}, the TC of B[a]P (>1) is relatively high, which implies that ryegrass has strong ability to transfer B[a]P. Ryegrass is easy to be planted and has strong biomass and pollution resistance. Ryegrass has certain potentials to be used as plant remediation materials of Pb-B[a]P polluted soils. In particular, ryegrass might show stronger remediation effects to low-concentration Pb (100mg·kg^{-1}) and B[a]P (30mg·kg^{-1}) polluted soils.

Pb and B[a]P mainly concentrate in root system of ryegrass, and there are a few contents in the shoots. Therefore, a certain range of Pb and B[a]P contaminated concentrations in soils could be absorbed by planting ryegrass. Ryegrass can absorb and remediate Pb-B[a]P combined polluted soils to some extent.

To discuss influences of Pb and B[a]P and their interaction on transfer and distribution of Pb and B[a]P in soil-ryegrass system, SPSS 23.0 software was used for a multiple linear regression analysis. In the multiple linear regression analysis, Pb and B[a]P contaminated concentrations were used as independent variables (x_1、x_2), while the contents of Pb and B[a]P in shoots and roots of ryegrass were used as dependent variables (y_{Pb}、$y_{B[a]P}$). Results are shown in Table 5.

It can be seen from Table 5 that under Pb-B[a]P combined pollution, Pb and B[a]P contents in shoots and roots of ryegrass are significantly positively correlated with Pb and B[a]P contaminated concentrations (p<0.01). It can be believed that the overall effect of regression equation is significantly and the overall linear relation between all independent variables (Pb and B[a]P contaminated concentrations) and dependent variables (Pb and B[a]P contents in shoots and roots of ryegrass). Influences of independent variables on dependent variables are significant, indicating that Pb and B[a]P contaminated concentrations influence the Pb and B[a]P contents in different parts of ryegrass significantly. The determination coefficient of Pb and B[a]P contaminated concentrations on B[a]P contents in shoots and roots of ryegrass is the highest (R^2=0.988, 0.956), while the determination coefficient of Pb and B[a]P contaminated concentrations on Pb contents in shoots is the lowest (R^2=0.896).

Effect of Pb-B[a]P combined pollution on the absorption of mineral elements in ryegrass. Pb-B[a]P combined stress influence N, P, K, Mg and Fe absorbency of ryegrass. Such influence can be divided into promotion and inhibition and degree of influence varies with category of elements. It can be seen from Table 6 that compared with the control, N, P, K, Mg and Fe contents decrease significantly with the increase of Pb and B[a]P contaminated concentrations. Under Pb-B[a]P combined stress, the N, P, K and Fe absorbency in the roots of ryegrass are higher than those in the shoots, while Mg contents in the shoots are significantly higher than those in the roots, indicating that root system of ryegrass are the main absorption and accumulation of N, P, K and Fe.

TABLE 5
The regression analysis on transfer and distribution of Pb and B[a]P in soil-ryegrass system

Different parts of ryegrass	Regression Equation	R^2	*p*
Shoots	y_{Pb}=-14.91 + 0.24x_1+0.07x_2	0.896	<0.01
	$y_{B[a]P}$=-2.59 + 0.45x_1 + 0.01x_2	0.956	<0.01
Roots	y_{Pb}=-12.70 + 0.37x_1 + 0.13x_2	0.939	<0.01
	$y_{B[a]P}$=-0.95 + 0.60x_1 + 0.01x_2	0.988	<0.01

TABLE 6
The effects of Pb-B[a]P combined pollution on the absorption of mineral elements in ryegrass(mg·kg^{-1})

Treatment	N		P		K		Mg		Fe	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
T1	102080.4a	114240.8a	165144.5a	293950.6a	98113.3a	196226.5a	3222.1a	1707.8a	837.1a	2512.7a
T2	106460.8a	111840.6a	131340.6b	245707.3b	96440.6a	186723.7a	3120.3b	1782.3a	849.8a	2810.2a
T3	96630.6b	104290.7a	119810.1b	202980.9b	92146.9a	184293.9a	3012.6b	1827.6a	845.6a	2911.7a
T4	97430.1b	108456.9a	121234.7b	218644.5b	88173.8b	132226.3b	3161.4b	1795.2a	872.9a	3012.9b
T5	91830.4b	108643.2a	128507.2b	224845.8b	89719.1b	173241.5a	3146.2b	1685.4b	832.2a	2819.3a
T6	71230.2c	80807.6b	118987.3b	217538.9b	87465.1b	127296.6c	2961.4c	1595.8b	762.6b	2612.2a
T7	77430.6c	81325.4b	111532.8b	206643.7b	84605.4b	132572.8b	2990.3c	1401.9c	743.7b	2511.5a
T8	74830.7c	82890.6b	103059.7b	208135.2b	81665.9b	163531.4a	2832.3c	1477.4c	723.7b	2727.2a
T9	74430.5c	82723.40b	106708.5b	192535.4b	84238.1b	123223.3c	2717.8c	1307.4c	621.5c	2239.6b
T10	73092.3c	81677.8b	105646.9b	191677.4b	86174.7b	135162.7b	2678.5c	1414.3c	651.4c	2465.2a
T11	62732.7d	91026.2c	78955.8c	10026.6c	86128.7b	146698.8b	2123.2d	1597.8b	673.4c	2614.5a
T12	60492.4d	90812.6c	77747.8c	10812.2c	76722.4c	126149.6c	2170.6d	1280.7c	652.7c	2259.3b
T13	52972.4e	61221.5d	67765.9d	9221.4c	76716.6c	134478.8b	2025.5d	1062.4d	584.8d	2133.5b
T14	51372.5e	61303.8d	65176.4d	8303.6d	70593.7c	117196.1b	1952.2d	1133.5d	557.5d	2067.9b
T15	57772.8e	72015.9e	53836.3e	8015.8d	69136.4d	151614.6a	2094.5d	1046.7d	526.2d	1934.6c
T16	46630.6f	54290.7f	29810.1f	6298.9e	67436.7d	158707.6a	1809.4e	733.5e	516.8d	1816.8c
T17	49435.7f	56430.6f	23120.4f	5276.9f	61229.7d	123286.3c	1834.7e	875.8e	502.4d	1751.1d
T18	46610.1f	55220.5f	16910.8f	5853.5f	59788.7e	155229.2a	1761.6e	864.4e	489.7e	1537.3e
T19	42080.9f	51240.7f	13130.3g	5177.3f	52097.1e	131904.5b	1623.3e	652.3f	484.4e	1472.7e
T20	45750.4f	40620.8g	10660.4g	4745.2g	49106.5f	117854.7d	1567.5f	593.6f	451.2e	1294.3f

TABLE 7
The regression analysis on absorption of N, P, K, Mg, Fe in ryegrass under Pb-B[a]P combined pollution

Different parts of ryegrass	Regression Equation	R^2	*p*
Shoots	y_N=94018.74 - 158.20x_1- 20.64x_2	0.872	<0.05
	y_P=141299.99 - 360.78x_1- 134.62x_2	0.953	<0.01
	y_K=96907.33 - 108.28x_1- 101.90x_2	0.948	<0.01
	y_{Mg}=3168.81 - 4.56x_1- 1.01x_2	0.903	<0.01
	y_{Fe}=843.36 - 1.07x_1- 0.82x_2	0.919	<0.01
Roots	y_N=107570.90 - 169.10x_1 - 82.74x_2	0.878	<0.05
	y_P=234641.79 - 782.12x_1+ 13.10x_2	0.736	>0.05
	y_K=155308.16 - 69.71x_1- 51.15x_2	0.156	>0.05
	y_{Mg}=1813.77 - 3.09x_1- 1.98x_2	0.938	<0.01
	y_{Fe}=2931.72 - 3.85x_1- 2.73x_2	0.912	<0.01

To discuss influences of Pb and B[a]P and their interaction on N, P, K, Mg and Fe absorption capacity of ryegrass, a multiple linear regression analysis was carried out by using SPSS 23.0 software. In the multiple linear regression analysis, Pb and B[a]P contaminated concentrations were used as independent variables (x_1、x_2), while N, P, K, Mg and Fe contents in shoots and roots of ryegrass were used as dependent variables (y_N、y_P、y_K、y_{Mg}、y_{Fe}). Results are shown in Table 7.

In Table 7, under Pb-B[a]P combined pollution, N contents in shoots and roots of ryegrass are significantly negatively correlated with Pb and B[a]P contaminated concentrations (p<0.05). P contents in shoots of ryegrass are significantly negatively correlated with Pb and B[a]P contaminated concentrations (p<0.01). P contents in roots are negatively correlated with Pb contaminated concentrations, and it is positively correlated with B[a]P contaminated concentrations (p>0.05). K contents in shoots show extremely significantly negative correlations with Pb and B[a]P contaminated concentrations (p<0.01), while K contents in roots are negatively correlated with Pb and B[a]P contaminated concentrations (p>0.05). Mg and Fe contents in shoots and roots show extremely significantly negative correlations with Pb and B[a]P contaminated concentrations (p<0.01). It can be believed that the overall effect of the regression is significantly, and the overall linear relation between independent variables (Pb and B[a]P contaminated concentrations) and dependent variables (N, Mg and Fe contents) are significant. Effect of independent variables on dependent variables is significantly. This reveals that Pb and B[a]P contaminated concentrations influence greatly N, Mg and Fe contents in different parts of ryegrass, and they exert extremely significant impacts on P and K contents in shoots of ryegrass. The determination coefficient of Pb and B[a]P contaminated concentrations on P and K contents in shoots of ryegrass are the highest (R^2=0.953, 0.948). The determination coefficient of Pb and B[a]P contaminated concentrations on K contents in roots of ryegrass is the lowest (R^2=0.156).

Mineral elements are essential vital factors for plant growth. Pb-B[a]P combined stress influence absorption of mineral elements of plants. In this study, N, P, K, Mg and Fe contents are decreased as a response to Pb-B[a]P combined stress, which might be caused by the inhibited mineral absorption of ryegrass under Pb-B[a]P combined stress. Under Pb-B[a]P combined stress, N, P, K and Fe contents in roots of ryegrass are higher than those in shoots, while Mg contents in shoots are significantly higher than those in roots. According to experimental results in this study, changes of mineral contents in ryegrass under Pb-B[a]P combined stress might be related with the regulation effect of Pb-B[a]P combined stress in absorption and transfer of different elements. N, P, K and Mg can move and then redistributed in plants. The shortage of N, P, K and Mg can redistribute mineral elements from roots to shoots [15]. Due to Pb-B[a]P combined stress, Fe contents in ryegrass becomes inadequate. However, Fe shortage occurs firstly in shoots of ryegrass, which is caused by the low mobility of Fe. This finally causes higher Fe contents in roots than those in shoots. Although K can move in ryegrass, our results demonstrate that K contents in roots are significantly higher than those in shoots. Pb and B[a]P contaminated concentrations are negatively correlated with K contents in ryegrass. This study indicates that Pb-B[a]P combined pollution may change cell membrane permeability and discharge small molecules containing K element.

CONCLUSIONS

In this study, the following observations emerged as noteworthy ones:

(1) Pb-B[a]P combined pollution increase Pb and B[a]P contents in ryegrass significantly. Low concentration of Pb (<100mg·kg^{-1}) and B[a]P are easy to accumulate in root system of ryegrass, while only few Pb and B[a]P migrate to shoots. Ryegrass achieves stronger remediation effect to low-concentration Pb (100mg·kg^{-1}) and B[a]P (30mg·kg^{-1}) combined pollution.

(2) Pb-B[a]P combined stress hinders mineral absorption of ryegrass. With the increase of Pb and B[a]P contaminated concentrations, N, P, K, Mg and Fe contents in ryegrass are decreased dramatically. The root system of ryegrass is the main absorption and concentration organ of N, P, K and Fe. Pb-B[a]P combined pollution is negatively correlated with N, K, Mg and Fe contents in roots and shoots.

ACKNOWLEDGEMENTS

This research was supported by NSFC (Natural Science Foundation of China (31600311, 31470398).

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Received: **02.02.2022**
Accepted: **25.03.2022**

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