Pol. J. Environ. Stud. Vol. 24, No. 1 (2015), 157-163
DOI: 10.15244/pjoes/22274

Original Research

Effect of Combined Pollution of Cd and B[a]P on Photosynthesis and Chlorophyll Fluorescence Characteristics of Wheat

Yue Li[1], Zhonglin Chen[1], Sunan Xu[1], Lihong Zhang[1]*, Wei Hou[1], Ning Yu[2]

[1]School of Environmental Science, Liaoning University, Shenyang 110036, PR China
[2]The Experiment Center of Environmental Monitoring, Shenyang, 110161, PR China

Received: 18 January 2014
Accepted: 30 April 2014

Abstract

The single and joint effects of cadmium(Cd) and benzo (a) pyrene (B[a]P) on the seedling growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.) were investigated after 4, 8, and 11 days of exposure under10 mg·L^{-1} Cd and 54 mg·L^{-1} B[a]P combined stress. In comparison with the control, the reductions under Cd + B[a]P stress treatment of Chl *a*, Chl *b*, Chl *a+b* and Chl *a/b* ratio were 53%, 44%, 49%, and 16% after 11-d exposure, the reductions under combination stress of *P*n, *G*s, *T*r, and *C*i were 61%, 72%, 67%, and 9%, the reductions of Fv/Fm, Φ_{PSII} and q_P under combination stress treatment were 24%, 23%, and 7% after 11 days, while the increases of *WUE* and q_N under Cd + B[a]P were 19% and 81% after 11-d exposure. The nonstomatal limitation is the major reason for the decrease of *P*n under the cadmium and B[a]P treatment in wheat leaves. The higher *T*r value in wheat leaves is probably a positive adaptation response to the cadmium and B[a]P. This adaption response may play a protective role in the photosystem, resulting in a higher *P*n. B[a]P may enhance the toxicity of the cadmium because they can penetrate into the perforated cells more easily. The toxicity of combined stress to photosynthesis and chlorophyll fluorescence parameters is stronger than the toxicity of single cadmium or single B[a]P, while cadmium had stronger toxic effects than B[a]P. The joint action of cadmium and B[a]P was a significant synergistic effect.

Keywords: *Triticum aestivum* L., Cd, B[a]P, photosynthesis, chlorophyll fluorescence

Introduction

The heavy metal cadmium (Cd) and benzo (a) pyrene (B[a]P) are ubiquitous in the environment, which are representative of inorganic and organic pollutants. Heavy metals and PAHs can enter the farmland system through the settlement way of atmospheric dust emissions and industrial emissions, oil, chemical fertilizers, irrigation, sewage sludge, coal combustion, and vehicle exhaust [1, 2]. They have adverse effects on not only crop growth and development, but also on threatening human and animal health through the food chain. Heavy metals and PAHs are the focus of environmental science research. The residue of B[a]P content of China Petroleum Wastewater Irrigation area in Shenyang and Fushun has been near 30 mg·kg^{-1}. The PAH-contaminated soil was up to 82 mg·kg^{-1} [3]. The low concentration (0.1 mg·L^{-1}) of B[a]P promoted seed germination of Maize, barley, and alfalfa. The high-concentration (10-100 mg·L^{-1}) PAHs have an inhibitory effect on plant growth [4, 5]. Plant biomass and plant height of pepper decreased significantly under B[a]P stress [6]. The root elongation of wheat, Chinese cabbage, and tomato showed significant inhibition effects under B[a]P stress [7-9]. The chlorophyll content of plants generally decreased under PAH stress. The reason for

---

*e-mail: lihongzhang132@163.com

decreased pigment content is that PAHs could hinder plant roots to absorb nutrients and water from the polluted soil, and lead to a decrease in pigment synthesis capacity [10].

There was a lot of research about the harm of cadmium on plants, which focused on the effects of plant growth and cell division, photosynthesis, stomatal function, enzyme activity, water relations, ion leakage, hormone metabolism, and so on [11-15]. The photosynthesis is the material basis of plant growth and yield formation, and there was a lot of research about the effect of cadmium on photosynthesis. Cadmium has a greater inhibitory effect on photosynthesis, but the inhibited reason has been controversial [16-18]. The inhibition of net photosynthetic rate and transpiration of clover, alfalfa, and soybean under cadmium stress are a linear relationship that suggests that the photosynthetic rate was mainly due to stomatal closure [19-22]. Cadmium leading to a decrease of net photosynthetic rate was due to stomatal resistance and mesophyll cells increasing CO_2 diffusion resistance [23]. Cadmium reduced PSII activity and photosynthesis mainly by suppressing its photosynthetic pigment content [24-26]. The reduction of photosynthesis is not consistent due to the different plant species, cadmium concentrations, and growth environment.

The chlorophyll fluorescence kinetics parameters contained important information about the process of photosynthesis, such as light energy absorption and transformation, energy transmission and distribution, reactivity, excess energy dissipation, and photoinhibition of photosynthesis and light-damaged state. Chlorophyll fluorescence parameters play a unique role in the detection of adverse effects on photosynthesis, can reflect the intrinsic photosynthetic characteristics, and are regarded as the internal probe to study the relationship between photosynthesis and the environment. In the resistant physiology, crop breeding and cultivation, plant ecology, chlorophyll fluorescence parameters have been widely used [27-30]. However, up to now no report about the effects of co-exposure to cadmium and B[a]P on photosynthesis and chlorophyll fluorescence in plants has been presented. In an attempt to study cadmium and B[a]P interactions on a species realistically subjected to potential metal and PAH exposures, we have chosen wheat as a test species. Wheat is one of the main food crops in China, and cadmium and B[a]P are common contaminants in industrialized areas where sewage irrigation, fertilizer application, and city gas result in stunted growth and yield reduction. For such a reason, the present work intends to explore the mechanisms of heavy metals and PAH interaction, employing cadmium and B[a]P as model xenobiotics. The present study aims at the effects and responses of cadmium and B[a]P on the photosynthetic characteristics and chlorophyll fluorescence in leaves of wheat seedlings of both single and combined pollution condition, attempts to reveal the non photochemical quenching parameters and the light energy distribution and the photochemical quenching of low PSII function, reveals the photosynthetic mechanism of physiological response mechanism of cadmium and B[a]P of wheat leaves, and provides the theoretical basis for food safety production and physiological mechanism interpretation of heavy metals and PAH pollution.

Materials and Methods

Plant Materials and Growth Conditions

$CdCl_2$ were purchased from the Wuhan Yuancheng Technology Development Co. Ltd., China. B[a]P (99% purity) were purchased from Fluka, Germany, and used without further purification. Other reagents used in the study were purchased from the Tianjin Kermel Reagents & Instruments Co. Ltd., China.

The variety of tested *Triticum aestivum* L. is Liaoning Spring No. 10. These wheat seeds were obtained from the Liaoning Dongya Seed Co., in Shenyang, China.

Wheat seeds were sterilized in 0.1% (v/v) $HgCl_2$ for 10 min, washed with tap water, and soaked in water one day. Then soaked seeds were sowed into Hoagland's solution (Hoagland and Arnon, 1950). Three seedlings of *Triticum aestivum* L. with a similar size, about 4 weeks old, and 6-8 cm height with 4-6 leaves were transplanted into each pot. The pollutant concentration of stress test are based on cadmium and B[a]P on *Triticum aestivum* L. growth inhibition rate to determine the preliminary experiments (data not shown). The initial concentration was set at 10 $mg \cdot L^{-1}$ Cd, 54 $mg \cdot L^{-1}$ B[a]P as moderate contamination in all treatments.

In glass beakers (1000 mL), 10 g of mineral substrate were mixed with 10 g of pre-washed perlite (agrilit) and 400 mL of a Hoagland solution at pH 6. The composition of the nutrient solution, expressed in $mmol \cdot l^{-1}$ was 30 KNO_3, 2.0 $Ca(NO_3)_2 \cdot 4H_2O$, 1.0 $NH_4H_2PO_4$, 0.5 $MgSO_4 \cdot 7H_2O$; and in $\mu mol \cdot l^{-1}$, 1. 25 Cl, 13 B, 1.0 Mn, 0.25 Cu, 0.25 Mo, and 10 Fe (supplied as ferric-sodium ethylene di-amine tetra acetate). The following treatments were employed:

(a) Complete Hoagland's nutrient solution
(b) Hoagland's solution with 10 $mg \cdot L^{-1}$ cadmium
(c) Hoagland's solution with 54 $mg \cdot L^{-1}$ B[a]P
(d) Hoagland's solution with 10 $mg \cdot L^{-1}$ cadmium and 54 $mg \cdot L^{-1}$ B[a]P.

Each treatment was prepared in triplicate and the position of pots was changed randomly every week. Leaves of wheat were taken at 0, 4, 8, and 11 days after treatment for the photosynthetic and chlorophyll fluorescence parameter analysis.

Chlorophyll Pigment Measurements

Chlorophyll pigments were soaked using 0.05 g of fresh material in 5 ml of 80% aqueous acetone. After filtering, 1 ml of the suspension was diluted with a further 2 ml of acetone and chlorophyll *a* (Chl *a*) and *b* (Chl *b*) contents were determined with a spectrophotometer using two wavelengths (663 and 645 nm). Concentrations of pigments ($\mu g \cdot g \cdot fwt^{-1}$) were obtained through calculation.

Gas Exchange Parameters

Net photosynthetic rate (*P*n), transpiration rate (*T*r), stomatal conductivity (*G*s), and intracellular CO_2 concentration (*C*i) were measured in clear morning from 8:30 to 11:30 a.m. using LI-6400 photosynthetic measurement systems (LI-COR, Lincoln, NE, USA) with the air temperature, relative humidity, CO_2 concentration, and light intensity inside the leaf chamber controlled at 25ºC, 55%, 370 $\mu mol\ CO_2 \cdot mol^{-1}$, and 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters of intact leaves (one leaf per plant, three plants per replicate) were measured using Li-6400-40LCF. The minimal chlorophyll fluorescence (*F*o) level when photosystem II centers are open was measured after applying a far-red pulse for 6 s. The maximal fluorescence (*F*m) after 60 min of dark adaptation was measured after applying a saturating flash for 0.8 s. Then the leaf was continuously illuminated with a white actinic light at a light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The steady-state value of fluorescence (*F*s) was reached within about 5 min and thereafter was recorded and a second saturating pulse at 8,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was imposed to determine the maximal fluorescence level in the light-adapted state (*F*m′). The actinic light was then removed and the minimal fluorescence level in the light-adapted state (*F*o′) was determined by illuminating the leaf with 4 s of far-red light. We calculated:

1) The maximal efficiency of PSII photochemistry in the dark-adapted state (*F*v/*F*m)
2) The photochemical quenching coefficient $q_P=(Fm'-Fs)/(Fm'-Fo')$ and nonphotochemical quenching coefficient $q_N=1-(Fm'-Fo')/(Fm-Fo)$
3) The actual quantum yield of PSII electron transport in the light-adapted state, $\Phi_{PSII}=(Fm'-Fs)/Fm'$

Statistical Analysis

All data were statistically analyzed by ANOVA using SPSS software package (SPSS version 18.0 for Windows, Chicago, IL, USA). ANOVA was performed to assess the main effect of each factor and their interactions. Means were separated by Duncan's multiple range test at 5% significance level.

Results

Effect of Cadmium and B[a]P on Chlorophyll Pigments Content

Cadmium, B[a]P, and their combination (Cd + B[a]P) reduced the pigments content of Chl *a*, Chl *b*, and Chl *a*+*b* over control treatment (Figs. 1 A, B, D). Compared to the control, the reductions under cadmium treatment of Chl *a*, Chl *b*, and Chl *a*+*b* were 15%, 21%, and 17% after 11-d exposure; the reductions under B[a]P treatment 8%, 10%, and 9%. Compared to the control, the reductions under combination stress treatment of Chl *a*, Chl *b*, and Chl *a*+*b* were 53%, 44%, and 49%. Compared to the control, the increments in both a/b ratio under cadmium, B[a]P treatment and their combination stress was 8%, 2%, 16% (Fig. 1 C). The gap of various chlorophyll contents was enlarged under the control in the 11th day compared with the 4th day.

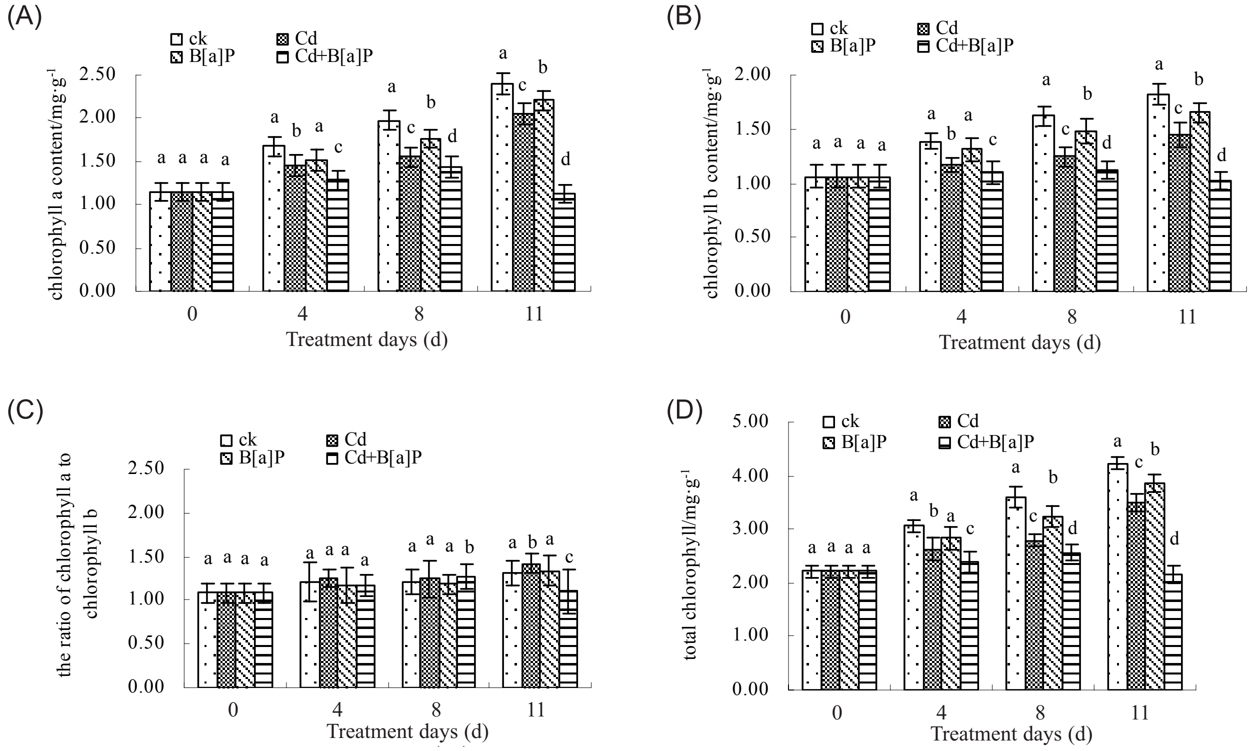

Fig. 1. Changes in chlorophyll pigments in the leaves of wheat seedling exposed to cadmium and B[a]P. (A) Chl *a* content; (B) Chl *b* content; (C) Chl *a/b* ratio; (D) Chl *a*+*b* content. Vertical bars represent standard deviations (n=3). The different letters indicate significant difference at $p<0.05$ (LSD multiple test).

The various treatments were significant difference ($p<0.05$). The combined stress has the greatest inhibitory effect on chlorophyll synthesis than cadmium and B[a]P stress.

Effect of Cadmium and B[a]P on Gas Exchange Parameters

Cadmium, B[a]P, and their combination (Cd+B[a]P) reduced *P*n, *G*s, *T*r, and *C*i over control treatment. Compared to the control, the reductions under cadmium treatment of *P*n, *G*s, and *T*r were 18%, 37%, and 33% after 11-d exposure, the reductions under B[a]P treatment 12%, 13%, and 15%, and the reductions under combination stress 61%, 72%, and 67% (Figs. 2 A, B, C). *P*n, *G*s, and *T*r under combination stress were lower than those under single stress. Compared to the control, the reductions of *C*i under cadmium, B[a]P, and Cd + B[a]P were 9%, 4%, and 9% after 11-d exposure (Fig. 2 D). Compared to the control, the increases of WUE under cadmium, B[a]P, and Cd + B[a]P were 23%, 3%, and 19% after 11-d exposure (Fig. 2 E).

Effect of Cadmium and B[a]P on Chlorophyll Fluorescence Parameters

In comparison with the control, the reductions of *F*v/*F*m under cadmium treatment, B[a]P treatment, and combination stress treatment were 13%, 6%, and 24% after 11 days (Fig. 3 A). Compared to the control, the increases

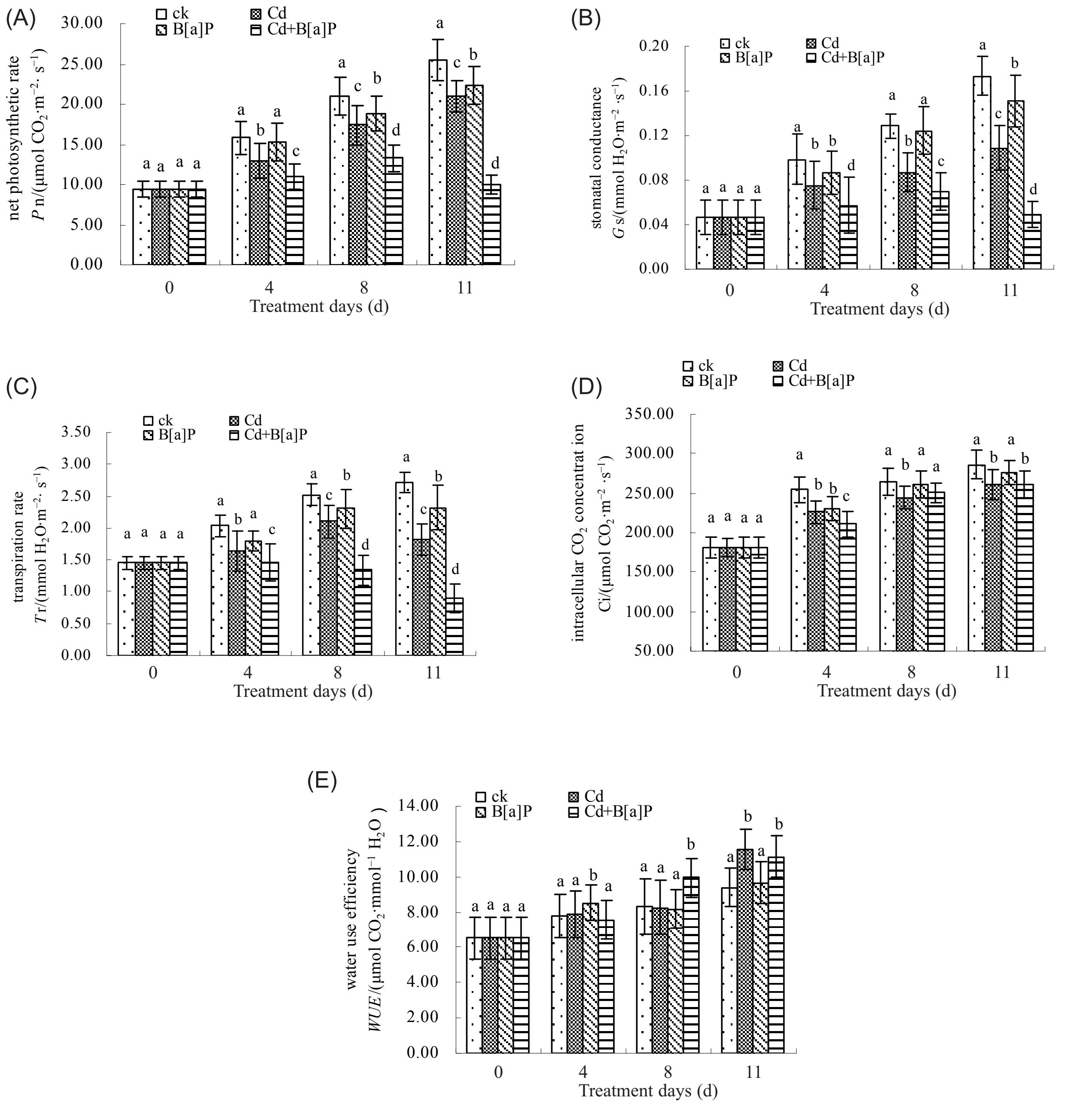

Fig. 2. Changes in gas exchange parameters in the leaves of wheat seedling exposed to cadmium and B[a]P. (A) Net photosynthetic rate, (B) Stomatal conduction, (C) Transpiration rate, (D) Intracellular CO_2 concentration, and (E) Water use efficiency. Vertical bars represent standard deviations (n=3). The different letters indicate significant difference at $p<0.05$ (LSD multiple test).

of Φ_{PSII} under cadmium treatment, B[a]P treatment and combination stress treatment were 20%, 9%, and 23% after 11 days (Fig. 3 B). Compared to the control, the reductions of q_P under cadmium treatment, B[a]P treatment, and combination stress treatment were 4%, 2%, and 7% after 11 days (Fig. 3 C). In comparison with the control, the increments of q_N under cadmium treatment, B[a]P treatment, and combination stress treatment were 59%, 49%, and 81% after 11 days (Fig. 3 D). The difference of combined stress and three treatments was significant ($p<0.05$). Cadmium and B[a]P have an inhibition effect on Fv/Fm and q_P, and combined stress is the most significant.

Discussion

Joint effects of pollutants may be similar (additive) or stronger (synergistic, more than additive), or weaker (antagonistic, less than additive) than expected effects from separate exposure. The effect of combined pollution depends on the constituents of the mixture and may vary significantly [31, 32].

Chlorophyll content is considered an indicator of damages to the photosynthetic system induced by environmental stressors [33, 34]. Our results showed that combined stress has the greatest inhibitory effect on chlorophyll synthesis than cadmium and B[a]P stress. Cadmium, B[a]P, and combination stress decreased chlorophyll contents. It is stated that hydrophobic pollutants such as PAHs interact with lipophilic compounds of cytoplasmatic membranes of microorganisms [35]. This could cause changes in the membrane structure and might alter the permeability of the membranes. Therefore, we assume that PAHs may enhance the toxicity of the metals because they can penetrate into the perforated cells more easily. In our experimental conditions, Cd + B[a]P elicited a more marked response than single cadmium and B[a]P, and therefore it is likely that synergistic effects may depend on the relative toxicity level of cadmium and B[a]P doses. Combined with the marked reduction in the Chl *a/b* ratio, this indicates that Chl *a* is more affected than Chl *b*. The reduction of chlorophyll contents indicates that cadmium and B[a]P hinder chlorophyll accumulation, and Chl *a/b* ratio under the cadmium and B[a]P stress is mainly to prevent the accumulation of Chl *b*.

Photosynthesis, as an important physiological process in plants, is the basis of the growth and development of plants, and was used as a bioindicator of early stress [36, 37]. Chlorophyll fluorescence emitted by higher plants can reflect photosynthetic activities in a complex manner [38], and it is widely used for analyzing the photosynthesis and related mechanisms in plants under biotic or abiotic stress [39, 40]. In this study a significant decrease of *P*n was

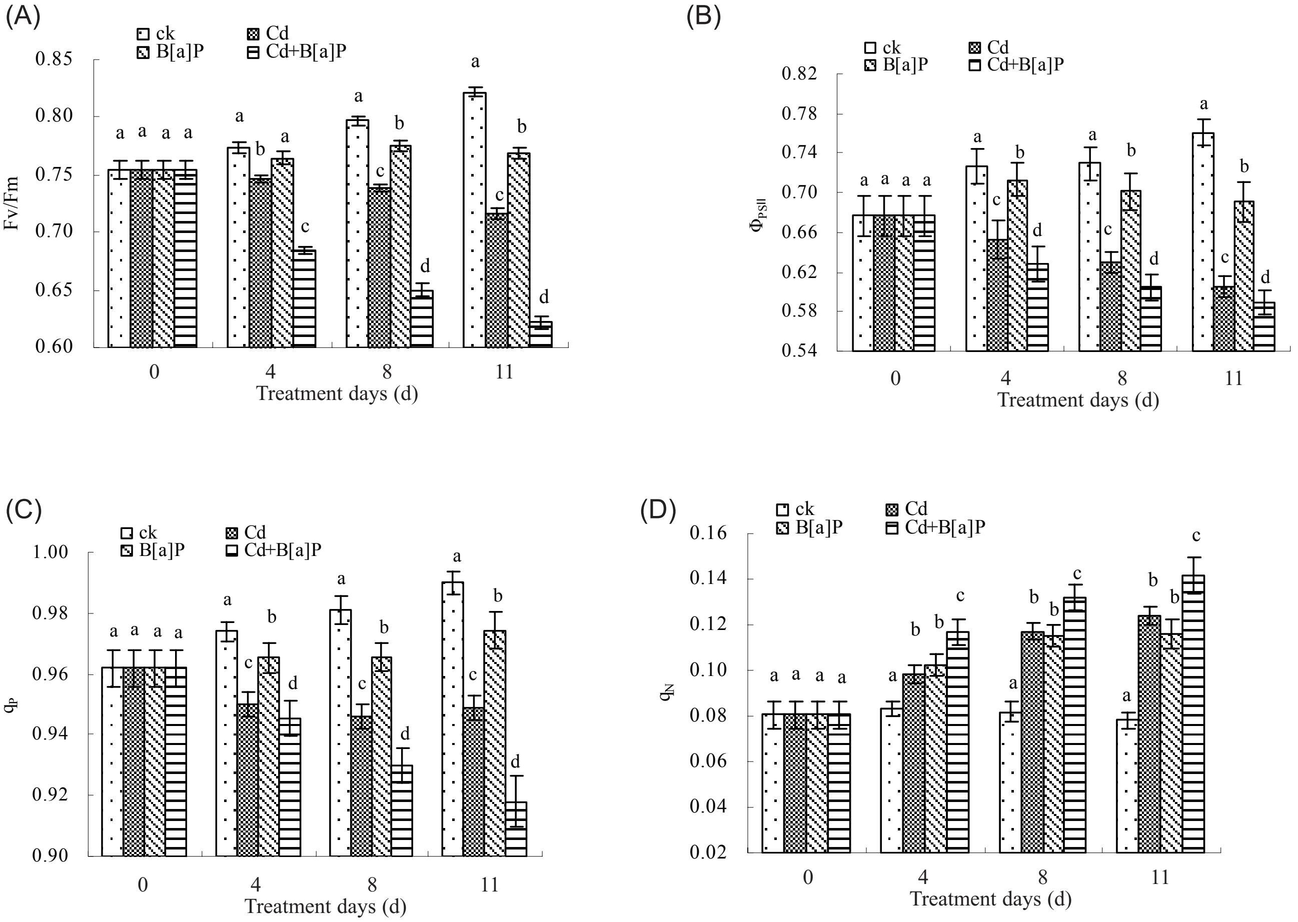

Fig. 3. Changes in chlorophyll fluorescence parameters in the leaves of wheat seedling exposed to cadmium and B[a]P. (A) Fv/Fm; (B) Φ_{PSII}; (C) q_P; (D) q_N. Vertical bars represent standard deviations (n=3). The different letters indicate significant differences at $p<0.05$ (LSD multiple test).

observed in wheat cultivars under cadmium and B[a]P treatment. *G*s was obviously reduced but no significant change occurred in *C*i in wheat seedlings, suggesting that cadmium and B[a]P was applied for photosynthesis in wheat leaves. When the reductions of *P*n and *G*s are associated with a decline of *C*i, stomatal factors are considered to be crucial in the decrease of photosynthetic rate; on the contrary, when the reductions of *P*n and *G*s are associated with the increase or stabilization of *C*i, the decrease of photosynthetic rate is primarily caused by nonstomatal limitation [41]. Therefore, nonstomatal limitation is the major reason for the decrease of *P*n under the cadmium and B[a]P treatment in wheat leaves. Besides, *T*r was also diminished by cadmium and B[a]P exposure, which was in relation to the decrease of *P*n and *G*s. The higher *T*r value in wheat leaves is probably a positive adaptation response to the cadmium and B[a]P. This adaption response may play a protective role in the photosystem, resulting in a higher *P*n. In addition to the nonstomatal limitation, the stomatal factor, with smaller effect, is also involved in the negative influence of cadmium and B[a]P on *P*n [41]. The wheat seedling leaves *P*n, *G*s, and *T*r combination stress is the greatest inhibitive than single cadmium and B[a]P stress. Cadmium is the greater inhibiter than B[a]P. The inhibition of photosynthesis seems to occur indirectly either through chlorophyll reduction or as a result of decreased stomatal conductance, as has been reported earlier [42].

*F*v/*F*m is the frequently used indicator of photoinhibition of PSII in response to stresses [43, 44]. We observed that *F*v/*F*m is decreased significantly under cadmium, B[a]P and combination stress. The change of q_N in chloroplast energy state is very sensitive, and this change is influenced by many factors, such as the environmental stress factor leading to stomatal closure, blocking the electron flow from CO_2 to O_2, and causing PSII energy conversion rate. Therefore, the q_N has been proved to be the most sensitive parameter for early detection of stress. Our results show that under the stress of Cd and B[a]P, q_N light response trends in contrast with q_P (Fig. 3 C, D). This showed under the stress of Cd and B[a]P, wheat seedlings avoid damaging photosynthesis systems by increasing heat dissipation. The results demonstrated that the photoreaction agency was injured, whereas the photo-protective capacity was increased. In a review recently issued by Gogolev and Wilke [45], it is stated that hydrophobic pollutants such as PAHs interact with lipophilic compounds of cytoplasmatic membranes of microorganisms.

Conclusion

In the present study it is certified that both cadmium and B[a]P had inhibitory effects on the chlorophyll pigments, gas exchange parameters, and chlorophyll fluorescence parameters of wheat seedlings. The toxicity of combined stress to chlorophyll pigments, gas exchange parameters, and chlorophyll fluorescence parameters is stronger than the toxicity of single cadmium or B[a]P, while cadmium had stronger toxic effects than B[a]P. The experimental data indicated that the toxicity of combined stress was higher than single cadmium and single B[a]P. Moreover, the variations in these physiological indexes of wheat could be considered as good biomarkers of serious stress by HM and PAHs in the environment.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31470398; 31270369; 31100349), the Project of the fifth teaching reform of Liaoning University (JG2013YB0021).

References

1. SUN F., ZHOU Q. Oxidative stress biomarkers of the polychaete Nereis diversicolor exposed to cadmium and petroleum hydrocarbons. Ecotox. Environ. Safe., **70**, (1), 106, **2008**.
2. XIA H. L., CHI X. Y., YAN Z. J., CHENG W. W. Enhancing plant uptake of polychlorinated biphenyls and cadmium using tea saponin. Bioresource Technol., **100**, (20), 4649, **2009**.
3. SUN Y. B., ZHOU Q. X., XU Y. M., WANG L., LIANG X. F. Phytoremediation for co-contaminated soils of benzo[a]pyrene (B[a]P) and heavy metals using ornamental plant *Tagetes patula*. J. Hazard. Mater., **186**, 2075, **2011**.
4. RAMOS R., GARCIA E. Induction of mixed-function oxygenase system and antioxidant enzymes in the coral *Montastraea faveolata* on acute exposure to benzo(a)pyrene. Comp. Biochem. Phys. C., **144**, (4), 348, **2007**.
5. XU X. H., XU D. F., WANG X. R., WU J. C., LIN R. Z. Biological responses of maize seedlings to single and combined stress of cadmium and phenanthrene. Environ. Sci., **32**, (5), 1471, **2011**.
6. HUANG H., LI S., GUO J. L. The Influence of cadmium (Cd^{2+}) to the antioxidant system and photosynthesis of seedling of *Zea mays* L. J. Agro-Env. Sci., **29**, (2), 211, **2010**.
7. CAI S. X., HE Y., QIU X. X., LAN Z. M. Effects of polycyclic aromatic hydrocarbon pyrene on growth, uptake of pyrene and other elements, nutritious qualities of Chinese cabbage. Chinese J. Soil Sci., **41**, (2), 452, **2010**.
8. LIU Y., ZHANG W., LIU M. D. Effect of cadmium-benzo(a)pyrene single and combined pollution on wheat seed germination. J. Agro-Env. Sci., **31**, (2), 265, **2012**.
9. SUN Y. B., XU Y. M., ZHOU Q. X., WANG L., LIN D. S., LIANG X. F. The potential of gibberellic acid 3 (GA_3) and Tween-80 induced phytoremediation of co-contamination of Cd and Benzo[a]pyrene (B[a]P) using *Tagetes patula*. J. Environ. Manage., **114**, 202, **2013**.
10. REILLEY K. A., BANKS M. K., SCHWAB A. P. Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. J. Environ. Qual., **25**, 212, **1996**.
11. LIN R. Z., WANG X. R., LUO Y., DU W. C., GUO H. Y., YIN D. Q. Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). Chemosphere, **69**, 89, **2007**.
12. LIU X., ZHANG S., SHAN X., CHRISTIE P. Combined toxicity of cadmium and arsenate to wheat seedlings and plant uptake and antioxidative enzyme responses to cadmium and arsenate cocontamination. Ecotox. Environ. Safe., **68**, (2), 305, **2007**.

13. JIN X., YANG X., ISLAM E., LIU D., MAHMOOD Q. Effects of cadmium on ultrastructure and antioxidative defense system in hyperaccumulator and non-hyperaccumulator ecotypes of *Sedum alfredii* Hance. J. Hazard. Mater., **156**, 387, **2008**.
14. LI W. C., WONG M. H. Interaction of Cd/Zn hyperaccumulating plant (*Sedum alfredii*) and rhizosphere bacteria on metal uptake and removal of phenanthrene. J. Hazard. Mater., **209**, 421, **2012**.
15. LU Z. W., ZHANG Z., SU Y., LIU C. F., SHI G. R. Cultivar variation in morphological response of peanut roots to cadmium stress and its relation to cadmium accumulation. Ecotox. Environ. Safe., **91**, 147, **2013**.
16. WAN X. Q., ZHANG F., XIA X. L., YIN W. L. Effects of cadmium on photosynthesis and chlorophyll fluorescence parameters of solution-cultured poplar plants. Sci. Silv. Sin., **44**, (6),73, **2008**.
17. LI L., Y L., SONG L. N., GU Y. J. Effects of cadmium stress on chlorophyll fluorescence parameters of *Lemna minor* L. Acta Sci. Cir., **30**, (5), 1062, **2010**.
18. CHEN L., LONG X. H., ZHENG X. T., LIU Z. P. Effect on the photosynthetic characteristics of Cd uptake and translocation in seedlings of two *Helianthus tuberosus* varieties. Acta Prata. Sin., **20**, (6), 60, **2011**.
19. ZHANG L. H., LI P. J., LI X. M., XU C. B. Effects of cadmium stress on the growth and physiological characteristics of wheat seedlings. Chinese J. Eco., **24**, (4),458, **2005**.
20. WANG M. E., ZHOU Q. X. Joint stress of chlorimuron-ethyl and cadmium on wheat *Triticum aestivum* at biochemical levels. Environ. Pollut., **144**, 572, **2006**.
21. TAO Y. M., CHEN Y. Z., LIANG S. C., LIANG Y. L. Physiological and biochemical properties of *Bruguiera gymnorrhiza* seedlings under cadmium stress. Chinese J. Eco., **27**, (5), 762, **2008**.
22. CAO Y., DUAN M., LIU Y. L., ZHAO T. H. Effects of NH_4^+-N on photosynthetic characteristics of Spring wheat plant under cadmium. Eco. Environ. Sci., **20**, (2), 359, **2011**.
23. SLYCKEN S. V., WITTERS N., MEERS E., PEENE A., MICHELS E., ADRIAENSEN K., RUTTENS A., VANGRONSVELD J., LAING D. G., WIERINCK I., DAEL V. M., PASSEL V. S., TACK F.M.G. Safe use of metal-contaminated agricultural land by cultivation of energy maize (*Zea mays*). Environ. Pollut., **178**, 375, **2013**.
24. CHEN C. H., ZHOU Q. X., BAO Y. Y., LI Y.N., WANG P. Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (*Triticum aestivum*). J. Environ. Sci., **22**, (12), 1966, **2010**.
25. RASCIO N., NAVARI-IZZO F. Heavy metal hyperaccumulating plants, How and why do they do it? And what makes them so interesting? Plant Sci., **180**, 169, **2011**.
26. SANGEETA Y., RAM C. Effect of heavy metals and phenol on bacterial decolourisation and COD reduction of sucrose-aspartic acid Maillard product. J. Environ. Sci., **25**, (1), 172, **2013**.
27. LU X. D., GAO Y. Z., LING W. T., ZHAO Y. H., LIU Z. X. Effects of polycyclic aromatic hydrocarbons on POD and PPO in *Lolium multiflorum* Lam. J. Agro-Env. Sci., **27**, (5), 1969, **2008**.
28. VINCENT-HUBERT F., ARINI A., GOURLAY-FRANCÉ C. Early genotoxic effects in gill cells and haemocytes of *Dreissena polymorpha* exposed to cadmium, B[a]P and a combination of B[a]P and Cd. Mutat. Res., **723**, 26, **2011**.
29. MARIA V. L., BEBIANNO M. J. Antioxidant and lipid peroxidation responses in *Mytilus galloprovincialis* exposed to mixtures of benzo(a)pyrene and copper. Comp. Biochem. Phys. C. **154**, 56, **2011**.
30. GOLAM J. A., WANG M., ZHOU Y. H., XIA X. J., MAO W. H., SHI K., YU J. Q. The growth, photosynthesis and antioxidant defense responses of five vegetable crops to phenanthrene stress. Ecotox. Environ. Safe., **80**, 132, **2012**.
31. PALANISAMI T., SEIDU M., MICHAEL B., MALLAVARAPU M., RAVI N. Microbial activity and diversity in long-term mixed contaminated soils with respect to polyaromatic hydrocarbons and heavy metals. J. Environ. Manage., **99**, 10, **2012**.
32. BAYEN S. Occurrence and bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems, A review. Environ. Int., **48**, 84, **2012**.
33. QIAN H. F., LI J. J., SUN L. W., WEI CHEN, G. SHENG D. E., LIU W. P., FU Z. W. Combined effect of copper and cadmium on *Chlorella vulgaris* growth and photosynthesis-related gene transcription. Aquat. Toxicol., **94**, 56, **2009**.
34. QIU Z. Y., WANG L. H., ZHOU Q. Effects of bisphenol A on growth, photosynthesis and chlorophyll fluorescence in above-ground organs of soybean seedlings. Chemosphere, **90**, 1274, **2013**.
35. TENG Y., SHEN Y. Y., LUO Y. M., SUN X. H., SUN M. M., FU D. Q., LI Z. G., CHRISTIE P. Influence of *Rhizobium meliloti* on phytoremediation of polycyclic aromatic hydrocarbons by alfalfa in an aged contaminated soil. J. Hazard. Mater., **186**, 1271, **2011**.
36. MOHAMMAD M., NAFEES A. K. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. J. Plant Physiol. **164**, 601, **2007**.
37. ZHANG Z. H., RENGELA Z., MENEY K., PANTELIC L., TOMANOVIC R. Polynuclear aromatic hydrocarbons (PAHs) mediate cadmium toxicity to an emergent wetland species. J. Hazard. Mater., **189**, 119, **2011**.
38. POKORA W, TUKAJ Z. The combined effect of anthracene and cadmium on photosynthetic activity of three *Desmodesmus* (Chlorophyta) species. Ecotox. Environ. Safe., **73**, 1207, **2010**.
39. TUKAJ Z., POKORA W. Individual and combined effect of anthracene, cadmium, and chloridazone on growth and activity of SOD izoformes in three *Scenedesmus* species. Ecotox. Environ. Safe., **65**, 323, **2006**.
40. ZHOU Y., ZOU X. L., CHEN G. C., SHAN Q. H., ZHANG J. F. Effect of phenanthrene on physiological characteristics of Salix jiangsuensis CL J-172 seedlings. J. Jiangxi Uni. Sci. Tec., **33**, (1), 1, **2012**.
41. FARQUHAR G. D., SHARKEY T. D. Stomatal conductance and photosynthesis. Annu. Rev. Plant Phys. **33**, 317, **1982**.
42. DONG J., WU F., ZHANG G. Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersicon esculentum*). Chemosphere, **64**, 1659, **2006**.
43. REDONDO-GOMEZ S., MATEOS-NARANJO E., ANDRADES-MORENO L. Accumulation and tolerance characteristics of cadmium in a halophytic Cd-hyperaccumulator, *Arthrocnemum macrostachyum*. J. Hazard. Mater., **184**, 299, **2010**.
44. CHIBUIKE C., LESLEY B. Effect of combined pollution of chromium and benzo (a) pyrene on seed growth of *Lolium perenne*. Chemosphere, **90**, 164, **2013**.
45. GOGOLEV A., WILKE B. M. Combination effects of heavy metals and fluoranthene on soil bacteria. Biol. Fert. Soils, **25**, 274, **1997**.