Plant Species Compositions Alleviate Toxicological Effects of Bisphenol a by Enhancing Growth, Antioxidant Defense System and Detoxification

Xianguang Nie

Ocean University of China - Laoshan Campus: Ocean University of China

Lin Wang (lwangouc@163.com)

Ocean University of China - Laoshan Campus: Ocean University of China https://orcid.org/0000-0002-3228-7574

Research Article

Keywords: Bisphenol A, plant species compositions, Antioxidant enzymes, Oxidative stress, Detoxification

Posted Date: June 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-510009/v1

Abstract

Bisphenol A (BPA), a broadly disseminated endocrine disturbing chemicals in environment, is harmful to creatures and plants. Plants can uptake and metabolize BPA, but a single plant species ability is limited. Undeniably, plant species compositions have a more vital ability to remove pollutants than a single plant species. However, the mechanisms of plant species compositions alleviating toxicological effects of bisphenol A are poorly understood. Here, we administered plant species compositions, which based on a full-factorial design of *Phragmites australis*(A), *Typha latifolia*(B) and *Arundo donax*(C), to unveil their role in BPA exposure. The comes about illustrated that with 1.5-10mg L^{-1} of BPA introduction, the mixed-hydroponic culture groups (e.g. sp(ABC)) significantly increased biomass production and photosynthetic pigments content as revealed by augmented the shoots fresh, dry weight, chlorophyll a and total chlorophyll content. While mixed-hydroponic culture groups (e.g. sp(AB), sp(ABC)) significantly increased antioxidant enzymes activity and antioxidant substances, it astoundingly diminished responsive oxygen species (ROS) and malondialdehyde (MDA) substance, proposing that mixed-hydroponic culture groups calmed oxidative stretch in takes off. Further analysis revealed that mixed-hydroponic culture groups (e.g. sp(AB), sp(AC), sp(ABC)) significantly increased detoxification enzyme activity of NADPH-cytochrome P450 reductase (CPR), glutathione S-transferase (GST) and glycosyltransferase (GT). Moreover, mixed-hydroponic culture groups (e.g. sp(AB), sp(AC), sp(ABC)) decreased the BPA substance in leaves, proposing that mixed-hydroponic culture groups advanced BPA metabolism by improving CPR, GST, GT detoxification. These results suggest that a mixed-hydroponic culture strategy can alleviate BPA phytotoxicity and possibly offer natural and potential phytoremediation of BPA way.

1. Introduction

Bisphenol A (Bisphenol A;2, 2-bis (4-hydroxyphenyl) propane) is used industrially to synthesize materials such as polycarbonate and epoxy resins (Staples et al., 1998). BPA is also applied in the production of many commodities, such as toys, water pipes, sports safety equipment, dental unit, medical equipment and pipelines, and electronics materials (Im and Loffler 2016, Wang et al., 2016). Although BPA can be covalently bound to these materials,when they are washed, heated, or come into contact with an acidic or basic pH, the molecules’ ester bonds are hydrolyzed, releasing the bisphenol A monomer into the environment (Liu et al., 2013). Due to the tall generation volumes and transfer of items made from BPA□BPA has brought about omnipresent within the environment (Maher et al., 2016, Wisniowska et al., 2020). Previous reports have repeatedly affirmed its presence in industrial and municipal effluents and sludge, as well as in fresh water (Lee et al., 2015).

In addition, the concentration of BPA was reported in landfill leachate of hazardous waste, reaching 17.2 mg L^{-1} (Yamamoto et al., 2001). Therefore, the BPA moves into the human body through the food chain so that human beings' health is threatened seriously. Previous studies have shown that BPA might cause several diseases such as childhood obesity, type II diabetes, developmental defects, cancer (Guida et al., 2015, Dumitrascu et al., 2020, Martinez et al., 2020).

Plant is an essential link in a food chain. Plants have the ability to take up BPA from their environment and translocate into above-ground parts, thus exerting severe influences on growth and development of plant (Nakajima et al., 2002).

A number of studies have uncovered the effects of BPA on plant growth, seed germination, photosynthesis, reproduction, reactive oxygen, heritability and antioxidant system in plants (Ferrara et al., 2006, Gattullo et al., 2012, Qiu et al., 2013, Ali et al., 2016, Zhang et al., 2016, Jiao et al., 2017, Ali et al., 2017, Xiang et al., 2018, Kim et al., 2018, Li et al., 2018). The antioxidant responses and “green liver” metabolic pathways are known for their responsiveness to BPA stress (Nakajima et al., 2002, Noureddin et al., 2004, Ali et al., 2016, Ahammed et al., 2020). BPA has been reported to affect the antioxidant system by reactive oxygen species (ROS), which were generated by oxidative stress of plant species (Li et al., 2008, Zhang et al., 2018). Exposure to BPA increased ROS, lipid peroxidation and antioxidant enzymes, which scavenge ROS (Ali et al., 2016, Xiang et al., 2018). “Green liver” metabolic pathways of plant have been shown to detoxify xenobiotics (Bartha et al., 2014, He et al., 2017). Several previous studies found that xenobiotic metabolism of BPA follows three processes in plant cells. To produce more water-soluble compounds, BPA was hydroxylated in phase I via cytochrome P450 (Sasaki et al., 2008, Nakamura et al., 2011, Yu et al., 2019). In phase II, the main product of phase I were conjugated with glycosides, via GT, and glutathione, via GST, to reduce the poisoning of plant cells (Nakajima et al., 2002, Shimoda and Hamada 2009, Ahammed et al., 2020). In phase III, the metabolites of phase II were compartmentalized into the vacuole. Therefore, it is necessary to conduct a complete mechanism study on the toxicity and the degradation pathway of BPA in plants.

Plants get energy from photosynthesis to absorb, retain and assimilate pollutants. However, there are few isolated single plant systems in nature, for in natural ecological environments multiple plant systems coexist (Fornara and Tilman 2008, Wang et al., 2020). Typically, the plants’ combining serves to rectify weaknesses of each constituent when it exists alone. A variety of plant combinations have a more vital ability to remove pollutants than a single plant species (Wang et al., 2016, Zhu et al., 2017). However, the plant species compositions alleviating toxicological effects of BPA have never been investigated in detail. This is because plant diversity improves the stability of ecosystem functions and enhances the effect of purifying pollutants (Zheng et al., 2016, Zhu et al., 2017). Therefore, exploring the physiological and biochemical states of various plants in various plant combinations is helpful to understand the mechanism of improving the purification efficiency of plant combinations.

Here, we picked out three macrophytes: *Phragmites australis* (A), *Typha latifolia* (B) and *Arundo donax* (C) who have the biotransformation and bioaccumulation capacity of environmental pollution (Bonanno 2013, He et al., 2017, Hamad 2020). Three macrophytes show a high morphological plasticity and maintain a significant capacity of pollutant removal in different environmental conditions (Calheiros et al., 2009, Zhu et al., 2017, Cao et al., 2019). This study aimed to reveal a suitable plant species composition method for carrying out physiological changes in aquatic plants exposed to BPA. We checked the compositions in terms of biomass production improvement, photosynthetic system pigment content, ROS, lipid peroxidation, antioxidant enzyme activity systems, antioxidant substances, and

detoxification enzymes. This study will help to understand the reaction mechanism of plant species compositions to BPA. We speculate that the alleviating effect of different plant combinations on BPA may vary with different BPA concentrations. The results of this study will provide a reference for the scientific evaluation of the ecological significance of plant species compositions and the risk of BPA pollution and formulating environmental control measures.

2. Materials And Methods

2.1. Plant cultivation and BPA treatment

The hydroponic experiment systems were set up in April 2019, which dimension of 22 × 22 × 26 cm and total volume was 12584 cm^3. Three common large aquatic plants in China, *Phragmites australis* (A), *Typha latifolia* (B), *Arundo donax* (C), were selected for the experiment. Based on a full-factorial design, three species were assembled into 7 plant combinations, including monocultures of each species (3 combinations) which were named sp(A)-(plant A, *Phragmites australis*), sp(B)-(plant B, *Typha latifolia*), sp(C)-(plant C, *Arundo donax*), all possible two-species mixtures (3 combinations) which were named sp(AB)-(plant A + B, *Phragmites australis* + *Typha latifolia*), sp(AC)-(plant A + C, *Phragmites australis* + *Arundo donax*), sp(BC)-(plant B + C, Typha latifolia + Arundo donax), and a three-species mixture of all species which is named sp(ABC)-(plant A + B + C, *Phragmites australis* + *Typha latifolia* + *Arundo donax*). The plant density was settled in each hydroponic box in diverse cultured types. Each hydroponic box contained 12 individual plants, evenly distributed (i.e., 1:1 in sp(AB)/ sp(AC) / sp(BC) or 1:1:1 in sp(ABC)), with each plant combination had 3 replicates (hydroponic box). In order to immobilize the pot and aquatic plants in the exposure medium, the commercially available stones were placed in each pot. All aquatic plant were allowed to acclimate in the hydroponic box of tap water for about ten days and then Hoagland medium ten days. The hydroponic experiment systems were fed with Hoagland medium, holding pH about 6.0. Next, each hydroponic box was filled with 10 L of Hoagland medium and supplemented with a total four concentrations of BPA (1.5, 5,10 and 20 mg L^{-1}), which was renewed every two days for ten days. The hydroponic test was ended at ten days after start of BPA treatment. Shoot of Plants were collected for the investigation of diverse biochemical parameters.

2.2 Determination of biomass and light harvesting pigments

fresh weights of shoots were measured and kept in an oven at 80°C for 96 h until a constant weight. The content of Chlorophyll a and total Chlorophyll pigments from leaves were measured according to a method described by Arnon (Arnon 1949). Fresh leaf samples (0.10 g) were placed in a 2 mL of absolute alcohol in dark. After 48 h,the ethanolic extracts were measured using UV–vis spectrophotometer (UV-2550, Shimadzu Corporation, Japan) and scanned at 645 and 663 nm for absorbance (OD). The gotten absorbance values were utilized to calculate Chlorophyll a and total Chlorophyll content.

$$Chlorophyll\ \ a\ \ \text{content} = 13.95 * OD663 - 6.88 * OD645$$
$$\text{total Chlorophyll content} = 20.2 * \text{OD645} + 8.02 * \text{OD663}$$

2.3 Assay of ROS levels and membrane lipid peroxidation

Superoxide anion (O_2^-) contents were measured according to previous methods (Elstner and Heupel 1976). The absorbance was recorded at 530 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan). Hydrogen peroxide (H_2O_2) contents were determined according to previous methods (Patterson et al., 1984). The absorbance was recorded at 412 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan). Hydroxyl radical (•OH) contents were determined according to previous methods with slight modification (Pandey et al., 2016). The absorbance was recorded at 532 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

The malondialdehyde (MDA) content was determined according to previous methods with slight modification (Heath and Packer 1965). The absorbance was recorded at 532 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

2.4 Determination of antioxidant enzyme activity

The fresh leaves (1.00 g) were homogenized in a 50 mM PBS (pH 7.8) containing 5 mM EDTA, 5 mM dithiothreitol and 1% (v/v) polyvinylpyrrolidone under ice cold conditions. The homogenates were centrifuged and the supernatants were used to perform following enzyme assays.

The superoxide dismutase (SOD) activity was assayed by according to Du (Du et al., 2015). The reaction mixture reacted about30 min in fluorescent lights. One unit of SOD activity was defined as the cause 50% inhibition of the NBT measured at 560 nm within 1 min (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

The peroxidase (POD) activity was measured by following Maehly and Chance (Maehly and Chance 1954). The reaction mixture (3 mL) contained 1 mL of 50 mM PBS (pH 6.8), 2% guaiacol, 2% H_2O_2, and 100 µL enzyme extract. The change of absorbance was recorded at 470 nm within 1 min (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

The activity of catalase (CAT) was assayed by according to previous methods with little modifications (Jiang and Zhang 2001). The reaction mixture contained 200 mM PBS (pH 7.8), 100 mM H_2O_2 and 50 µL of enzyme extract. The change of absorbance was recorded at 240 nm within 1 min (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

2.5 Determination of non-enzymatic antioxidant substance content

The ascorbic acid (AsA) content was measured in accordance with previous methods (Singh et al., 2006). The fresh leaves (0.20 g) were homogenized in 5% TCA and centrifuged. The reaction mixture stored 0.1 mL supernatant, 0.9 mL PBS (pH 7.4) and 1mL deionized water. The absorbance was recorded at 525 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

The Glutathione (GSH) content was assayed by according to previously methods with slight modification (Devos et al., 1992). The fresh leaves (0.20 g) were homogenized in 5% TCA and centrifuged 15 min at 4°C. The reaction mixture contained 0.1 mL supernatant, 2.6 mL PBS (pH 7.7) and 0.18 mL 5, 5-dithiobis-(2-nitrobenzoic acid). The absorbance was recorded at 412 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

The free proline (Pro) content was estimated according to previously methods with slight modification (Troll and Lindsley 1955). The fresh leaves (0.20 g) were homogenized in 10 mL of 3% sulfosalicylic acid and centrifuged for 10 min at 4°C. The reaction mixture contained supernatant, ice acetic acid and 2.5% ninhydrin (1:1:1 V/V). The absorbance was recorded at 520 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan).

2.6 Determination of detoxification enzyme activity

The microsomes are extracted according to previously reported with slight modification (Tan et al., 2015). The fresh leaves about 5 g were homogenized in 2 volumes of 50 mM PBS (pH 7.8) and then was centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was centrifuged at 100,000× *g* for 60 min and then the pellet was resuspended in 100 mM PBS (pH 7.8), containing 25% (v/v) glycerol and 10 mM mercaptoethanol.

The NADPH-cytochrome P450 reductase (CPR) activity was determined by the method described previously with slight modification (Guengerich et al., 2009). The reaction mixture (2 mL) contained 0.05 mL microsomal suspension, 5 mg mL^{-1} cytochrome c, 50 mM PBS (pH 7.8), 10 mM NADPH. The absorbance was recorded at 550 nm (UV-2550 spectrophotometer, Shimadzu Corporation, Japan). The enzyme activity was expressed as nmol min^{-1} mg^{-1} protein using a millimolar extinction coefficient of 21.1 cm^{-1}.

The glutathione S-transferase (GST) activity was determined by a modified protocol as described previously (Fuerst et al., 1993). The fresh leaves (about 1.00 g) were homogenized in 100 mM PBS (pH 7.8) and centrifuged for 10 min at 4°C. The reaction mixture (2 mL) contained 0.05 mL supernatant, GSH, 1-Chloro-2,4-dinitrobenzene and PBS (pH 7.8). The enzyme activity was expressed as nmol min^{-1} mg^{-1} protein using a millimolar extinction coefficient of 9.6 cm^{-1}.

The glycosyltransferases (GT) activity was determined according to previously methods with slight modification (Zhang et al., 2017). The fresh leaves (about 1.00 g) were homogenized in 100mM PBS (pH 7.8) and centrifuged at 4°C for 30 min. The reaction mixture was contained 100 µL supernatant, UDP-glucose and p-nitrophenol. The reaction mixture was added with 250 µL methanol and chilled at − 20°C for 0.5 h. The reaction mixture was analyzed using high performance liquid chromatography (LC-20A HPLC, Shimadzu Corporation, Japan) with a ultraviolet (UV) detection. One unit of the GT activity was defined as the consumption of 1µmol p-nitrophenol per minute.

2.7 Measurement of BPA content

Leaves of BPA content was estimated by high performance liquid chromatography (HPLC) as described previously with slight modification (Loffredo et al., 2010). The fresh leaves were dried until a constant weight. The dried leaves (1.00 g) were homogenized in 5 mL methanol and shaken on an oscillator for 4 h. The supernatant was evaporated off using a rotary vacuum evaporator (40°C). The residual product was homogenized in 5 mL of 60% (v/v) acetonitrile. The supernatant was filtered through a 0.45 µm Millipore™ filters and analyzed using HPLC (LC-20A, Shimadzu Corporation, Japan) with a ultraviolet (UV) detection under the condition: Inertsil ODS-3 column (4.6 × 250 mm, 5 µm), 217 nm wavelength, 70% methanol mobile phase, 0.6 mL min^{-1} flow rate, 20 µL of the injection volume. Residual BPA content was calculated by standard curve which uses BPA samples with known concentration.

2.8 Statistical analysis

All assays were conducted in triplicate replications. The results were expressed as the mean ± standard deviation. Treatment groups and control were analyzed by variance (ANOVA); $p < 0.05$ was considered statistically significant (SPSS 22.0, IBM).

3. Results And Discussion

3.1 mixed-hydroponic culture groups improve biomass production

After 10 d of exposure to BPA, variation of the shoots fresh and dry weight of seven cultured groups of the single species seedlings were observed in Fig. 1. With increase of BPA concentration, the shoots fresh and dry weight were increased first and then decreased respectively as compared to 0 mg L^{-1} BPA treatment. This phenomenon is in agreement with previous studies that BPA concentrations has a cytokinin-like effect, inducing plant cell elongation and proliferation, thereby promoting plant growth to a certain extent (Li et al., 2018, Xiao et al., 2019). Concentrations of BPA even destroyed the cell structural integrity (Ali et al., 2016, Kim et al., 2018). The shoots fresh and dry weight existed significant differences from1.5 mg L^{-1} to 10 mg L^{-1} BPA concentrations, respectively as compared to single species control (sp(A), sp(B), sp(C)). Consequently, the shoots fresh and dry weight of sp(ABC-A), sp(ABC-B) and sp(ABC-C), which three plant species compositions formed sp(ABC), were significantly increased in 1.5 mg L^{-1} to 10 mg L^{-1} concentrations of BPA. In detail, the shoots fresh and dry weight of sp(ABC-A), sp(ABC-B),sp(ABC-C) were increased by a maximum of 11.66 and 21.21%, 7.90 and 22.01%, 8.31 and 21.38%, respectively. This result recommends that upgrading plant species diversity will result in utilizing more pollutants for higher biomass production (Gross 2008, Fornara and Tilman 2009, Zhu et al., 2017).

3.2 Effect on chlorophyll a and total chlorophyll content

Chlorophyll plays a pivotal role in light capture and photosynthesis (Wang et al., 2020, Wang et al., 2020). It traps light energy and provides reducing power for carbon assimilation. To further explore the effect of BPA on chlorophyll, chlorophyll a and total chlorophyll content in seedlings leaves were determined in

Fig. 2. The chlorophyll a and total chlorophyll content were increased first and then decreased with increasing BPA concentration. Several studies have been reported in soybean and Vigna radiata chlorophyll content induced by bisphenol A (Qiu et al., 2013, Kim et al., 2018). It may be speculated that low concentration of BPA leaded to hormesis phenomenon. High concentration of BPA increased the accumulation of ROS, which have damaged the pigments and interfered key enzyme in chlorophyll synthesis (Qiu et al., 2013, Jiao et al., 2015, Jiao et al., 2017). Chlorophyll a and total chlorophyll content of sp(AB-A), sp(AC-A), sp(ABC-A), sp(AB-B), sp(ABC-B), sp(AC-C) and sp(ABC-C) were significantly higher from 1.5 mg L^{-1} to 10 mg L^{-1} BPA concentrations, as compared to single species control(sp(A), sp(B), sp(C)) respectively. Chlorophyll a content of sp(AB-A), sp(AC-A), sp(ABC-A), sp(AB-B), sp(ABC-B), sp(AC-C) and sp(ABC-C) were increased by a maximum of 14.07, 13.01, 14.89, 19.44, 19.93, 15.80, 18.95%, respectively, and total chlorophyll content were increased by a maximum of 11.39, 11.98, 11.26, 17.42, 16.98, 13.96, 13.23%, respectively. It follows that three cultured groups of sp(AB), sp(AC) and sp(ABC) were increased in chlorophyll a and total chlorophyll content. These results suggest that plant compositions improved stress tolerance and delayed chlorophyll degradation.

3.3 plant compositions reduced ROS accumulation and lipid peroxidation

Environmental stress can produce the reactive oxygen species (ROS), which is residual products of various categories metabolic pathways in plant cell (Ali et al., 2017). ROS accumulation exceed the antioxidant scavenging capacity and created oxidative stress in chloroplasts, plasma membrane, mitochondria and peroxisomes (Biczak et al., 2017). Malonaldehyde (MDA) characterizes the oxidative damage to lipid membranes to plants (Ali et al., 2016). Figure 3 shows the endogenous levels of O_2^-, H_2O_2, •OH and MDA content in all large aquatic plants exposed to different concentrations of BPA. The levels of O_2^-, H_2O_2, •OH and MDA contents in the leaves were increased with the increase BPA concentration. Increases in levels of ROS indicate that the presence of BPA triggered oxidative stress responses and lead to adding MDA content and lipid peroxidation (M. Dogan 2010, Wang et al., 2015, Zhang et al., 2016, Pawlowska et al., 2019). ROS may be responsible for inhibiting biomass and making chlorophyll degradation. The levels of O_2^-, H_2O_2, •OH and MDA contents of all single species of sp(AB), sp(AC) and sp(ABC) groups significantly lower than single species control (sp(A), sp(B), sp(C)), respectively, from 5 mg L^{-1} to 10 mg L^{-1} BPA exposure. Compared to sp(A), sp(B), sp(C), the O_2^-, H_2O_2, ·OH levels and MDA contents of sp(AB-A), sp(AB-B), sp(AC-A), sp(AC-C), sp(ABC-A), sp(ABC-B) and sp(ABC-C) were remarkably decreased in 5 mg L^{-1} to 10 mg L^{-1} BPA. In summary, the levels of O_2^-, H_2O_2, •OH and MDA contents were all relieved stress in sp(AB), sp(AC) and sp(ABC) group. For example, the levels of O_2^-, H_2O_2, •OH and MDA contents of sp(AB-A) and sp(AB-B), which make up sp(AB), were reduced by a maximum of 13.69, 28.16, 8.66, 6.21% and 10.83, 29.22, 13.33, 6.13%, respectively. The levels of O_2^-, H_2O_2, •OH and MDA contents of sp(ABC-A) and sp(ABC-B) and sp(ABC-C), which make up sp(ABC), were reduced by a maximum of 14.90, 28.18, 10.56, 7.19% and 11.36, 36.68, 14.10, 7.18% and

10.39, 27.69, 12.92, 4.37%, respectively. It indicates that levels of O_2^-, H_2O_2, •OH and MDA contents were relaxed and relieved stress by mixed-hydroponic culture groups. It is well known that ROS (especially •OH) participate in the degradation of BPA and decrease BPA (Wang and Lim 2011, Reis et al., 2014). On some certain conditions, ROS can be converted to each other (Mattila et al., 2015). Hence, this relieved phenomenon was probably correlated with the production of ROS (especially •OH) by biological Fenton reaction, Haber-Weiss reactions and antioxidant enzyme reaction (Halliwell 1999, Reis and Sakakibara 2012). Similar observed effects were reported by previous studies (Wang et al., 2015, Inagaki et al., 2016, Zhang et al., 2018).

3.4 Effect of BPA on antioxidant enzymes activities

To defend plant organism from oxidative stress, plants have an antioxidant defense mechanism that can scavenge ROS in cell (Xiao et al., 2020). The production and cleanup of ROS exists in homeostasis in vivo, the excessive ROS can damage the plant organism (Czarnocka and Karpinski 2018). Hence, antioxidant enzymes play a key role in clearing up excess ROS or changing them, which include SOD, POD and CAT. Protecting cells from O_2^- toxicity, SOD catalyzes the disproportionation of O_2^- to O_2 and H_2O_2. H_2O_2 are further converted to non-toxic oxygen and water by CAT and POD (Xu et al., 2008). In order to investigate the response of all large aquatic plants to BPA stress, the activities of SOD, POD and CAT in leaves were measured (Fig. 4). It was found that SOD, POD and CAT activities were increased after BPA exposure. This result indicated that BPA stress induced an enhanced in antioxidant enzymes activities, which effectively eliminated ROS to protect normal physiological functions of plant. In comparison to single species control (sp(A), sp(B), sp(C)), the SOD, POD and CAT activities of all single species of sp(AB), sp(AC), sp(BC)and sp(ABC) groups were significantly increased after 10d of 5 mg L^{-1} and 10 mg L^{-1} BPA exposure. For example, the SOD, POD, CAT activities of sp(AB-A) and sp(AB-B), which formed sp(AB), were significantly increased by a maximum of 13.63 and 14.35%, 32.68 and 28.00%, 31.03 and 71.98%, compared with single species control(sp(A) ,sp(B)), respectively. Meanwhile the SOD, POD, CAT activities of sp(ABC-A), sp(ABC-B) and sp(ABC-C), which three plant species compositions formed sp(ABC), were significantly increased by a maximum of 12.91, 13.72 and 40.16%, 36.08, 29.57 and 45.21%, 34.53, 64.01 and 77.22%, compared to single species control (sp(A), sp(B), sp(C)), respectively. These observations suggest that SOD, POD and CAT activities were further enhanced by mixed-hydroponic culture groups. This result indicated that antioxidant enzymes activities further effectively eliminated ROS to increase biomass and inhibit chlorophyll degradation of by mixed-hydroponic culture groups.

3.5 Effects of BPA on the content of antioxidant substances

Ascorbic acid (AsA) has antioxidant functions, which is an effective scavenger for the hydroxyl radicals and superoxide (Li et al., 2020). Glutathione (GSH) is a key antioxidant copiously distributed in plants and animals (Ma et al., 2019, Ahammed et al., 2020). GSH is a low-molecular-weight thiol, which can directly remove ROS (Geu-Flores et al., 2011). At the same time, GSH is also involved in the detoxification of

xenobiotics (Ahammed et al., 2020). Free proline (Pro) regulates cell membranes osmosis and responses to salinity, drought and other osmotic environmental stresses (Stein et al., 2011). Figure 3 depicts the effects of BPA on antioxidant substances in all large aquatic plants leaves. With increase the concentration of BPA, the ASA, GSH and Pro contents were increased in leaves. This result indicated that BPA stress induced an increase in antioxidant substances contents, which effectively eliminated ROS and reduced BPA contents and mediated osmotic adjustment in leaves. The ASA, GSH and Pro contents of the single species of sp(AB), sp(AC), sp(BC) (without GSH) and sp(ABC) cultured groups were significantly increased from 5 mg L^{-1} to 10 mg L^{-1} BPA, as compared to single species control (sp(A), sp(B), sp(C)), respectively. For example, the ASA, GSH, Pro contents of sp(AB-A) and sp(AB-B), which formed sp(AB), were significantly increased by a maximum of 44.09 and 54.35%, 16.43 and 13.11%, 24.43 and 76.22%, compared with single species control (sp(A), sp(B)), respectively. Meanwhile the ASA, GSH, Pro contents of sp(ABC-A), sp(ABC-B) and sp(ABC-C), which three plant species compositions formed sp(ABC), were significantly increased by a maximum of 54.12, 53.89 and 40.52%, 16.03, 15.07 and 12.53%, 28.16, 73.67 and 50.99%, compared to single species control (sp(A), sp(B), sp(C)), respectively. These observations indicated that the ASA, GSH and Pro contents were further enhanced by mixed-hydroponic culture groups. These results suggest that antioxidant substances contents further effectively eliminated ROS reduced BPA contents and mediated osmotic adjustment for protecting normal physiological functions by mixed-hydroponic culture groups.

3.6 Effect of BPA dosage on detoxification enzyme activity

NADPH-cytochrome P450 reductase (CPR) is part of cytochrome P450s system, which transfers the reduced xenobiotics from NADPH to the cytochrome P450 (Chen et al., 2021). glutathione S-transferase (GST)and glycosyltransferase (GT) catalyzed conjugation of toxicants with GSH and sugar, respectively, and the complex were further delivered to subcellular apartment for catabolism (Zhang et al., 2017, Chen et al., 2021). Figure 4 illustrates the effects of BPA dosage on CPR, GST, GT activities and BPA contents in seven cultured groups of the single species seedlings leaves. Previous studies show that BPA was catalyzed the hydroxylation, epoxidation by cytochrome P450s system in phase I (Hamada et al., 2002, Sasaki et al., 2005, Gabriel et al., 2007). In phase II, BPA or BPA- hydroxylated was catalyzed synthesis reactions with GSH and sugar by GST and GT (Nakajima et al., 2002, Nakajima et al., 2004, Kanwar et al., 2020). Hence, the activities of CPR, GST, GT and BPA contents in leaves were increased with increasing BPA dosage, implying that BPA-degraded and residual BPA content were closely related to the enhanced plant detoxification of the enzymes. The CPR, GST and GT activities of all the single species of sp(AB), sp(AC), sp(BC) (only in 1.5 mg L^{-1} BPA) and sp(ABC) cultured groups were significantly increased from 1.5 mg L^{-1} to 10 mg L^{-1} BPA, respectively as compared to single species control (sp(A), sp(B), sp(C)), respectively. For example, the CPR, GST, GT activities of sp(AB-A) and sp(AB-B), which formed sp(AB), were significantly increased by a maximum of 34.32 and 59.16%, 22.88 and 15.66%, 65.51 and 55.79%, compared with single species control (sp(A), sp(B)), respectively. Meanwhile the CPR, GST, GT activities of sp(ABC-A), sp(ABC-B) and sp(ABC-C), which three plant species compositions formed sp(ABC), were

significantly increased by a maximum of 35.39, 75.38 and 74.98%, 21.55, 16.04 and 33.41%, 69.47, 64.73 and 53.40%, compared to single species control, respectively.

We also measured BPA contents in leaves. The BPA contents of all the single species of sp(AB), sp(AC) and sp(ABC) cultured groups were significantly decreased from 1.5 mg L^{-1} to 10 mg L^{-1} BPA, respectively as compared to single species control (sp(A), sp(B), sp(C)), respectively. For example, the sp(AB-A),sp(AB-B)sp(AC-A), sp(AC-C), sp(ABC-A), sp(ABC-B) and sp(ABC-C), which formed sp(AB), sp(AC) and sp(ABC) cultured groups, were significantly decreased by a maximum of 28.38, 12.61, 25.82, 24.14, 33.30, 15.20 and 30.33%, compared with single species control (sp(A), sp(B)), respectively. These results suggest that BPA contents were further effectively reduced by mixed-hydroponic culture groups in leaves.

4. Conclusions

In conclusion, we found that BPA can be harmful to Phragmites australis(A), Typha latifolia(B) and *Arundo donax*(C) through the induction oxidative stress in leaves, which eventually inhibited biomass production and chlorophyll content. However, the mixed-hydroponic cultures (sp(AB), sp(ABC)) alleviate toxicological effects of BPA. This conclusion is supported by a proposed model depicting of the plant species compositions (Fig. 5). Firstly, BPA contents were further reduced by biotransformation and degradation of detoxification enzymes and biological Fenton reaction, Haber-Weiss reaction in the mixed-hydroponic cultures (sp(AB), sp(ABC)). BPA induced oxidative stress ability was severely weakened. Secondly, ROS levels which were produced by oxidative stress of BPA were further lowered by antioxidant enzymes and antioxidant substances content in the mixed-hydroponic cultures (sp(AB), sp(ABC)). Thirdly, biomass production reduction and chlorophyll degradation were relieved due to the reduction of ROS levels in the mixed-hydroponic cultures (sp(AB), sp(ABC)). These results have already illustrated that reasonable plant richness and sort play an vital part in alleviating BPA stress. This study gives valuable data on how to create artificial floating island and constructed wetland with tall working BPA expulsion.

Declarations

Author information

Affiliations

College of Environmental Science and Engineering, Ocean University of China, Qingdao, 266100, China

Xianguang Nie & Lin Wang

Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Xianguang Nie. The first draft of the manuscript was written by Xianguang

Nie; all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Corresponding authors

Correspondence to Lin Wang.

Funding

This work was supported by the Program of China Scholarship Council (No. 20120370391) and the National Key Research and Development Program of China (NO. 2018YFC0408000, 2018YFC0408004).

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

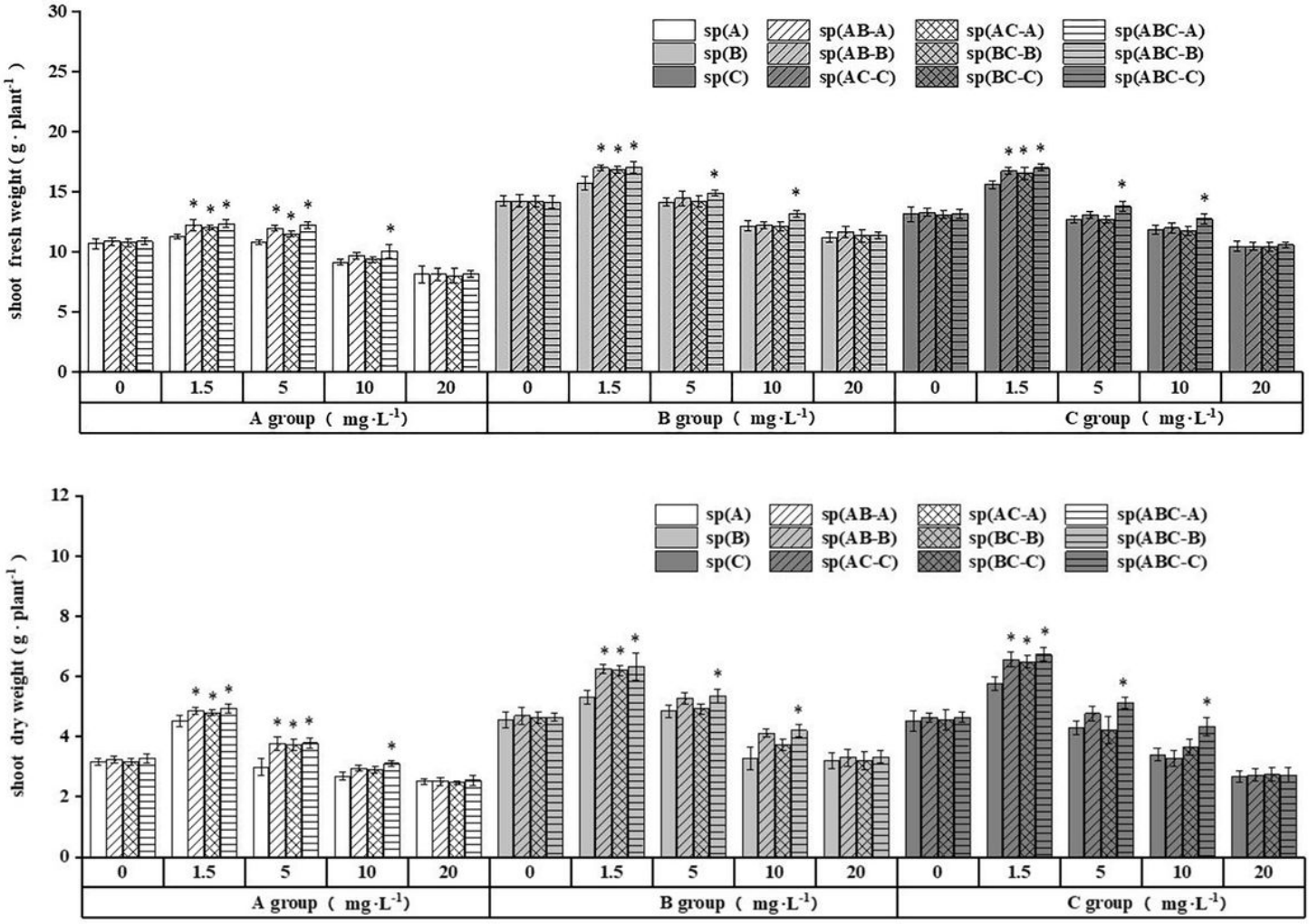

Figure 1

Effects of BPA on the shoots fresh and dry weight after ten days of exposure. The error bars indicate standard deviation (n=3). The asterisk (*) indicates significant differences ($p<0.05$) with compared to single species control (sp(A), sp(B), sp(C)), respectively.

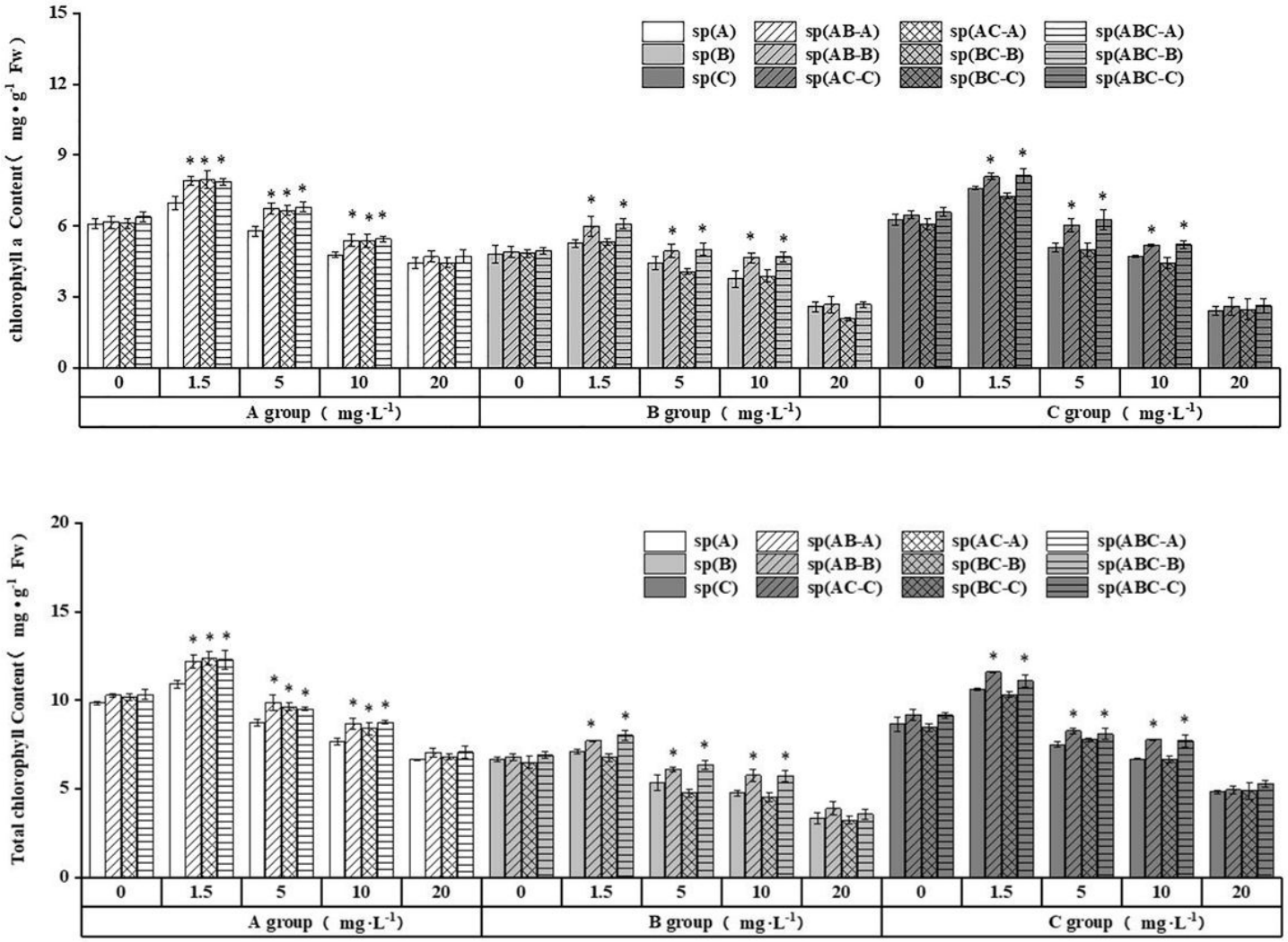

Figure 2

Effects of BPA on chlorophyll a and total chlorophyll content of leaves after ten days of exposure. The error bars indicate standard deviation (n=3). The asterisk (*) indicates significant differences ($p<0.05$) with compared to single species control (sp(A), sp(B), sp(C)), respectively.

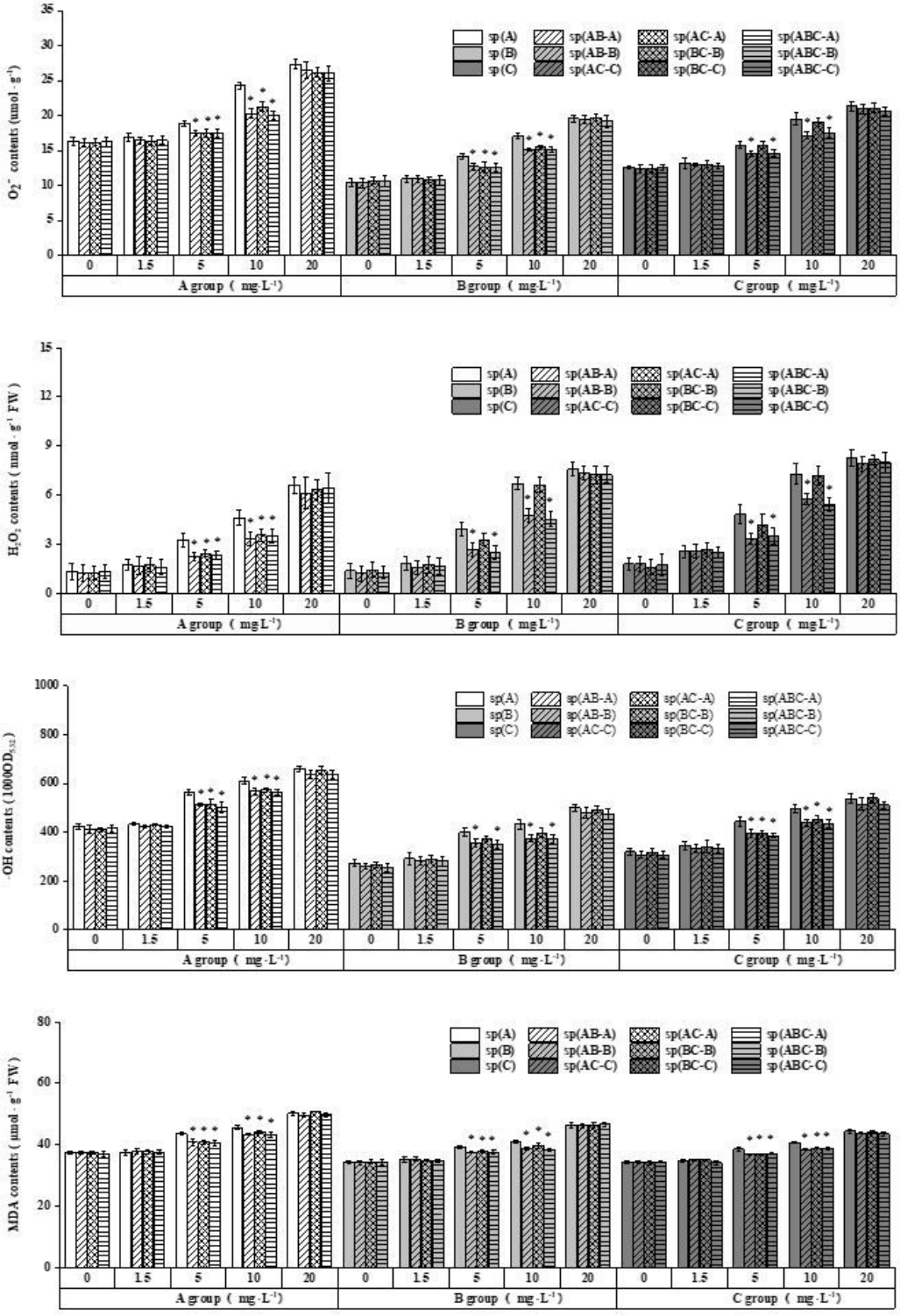

Figure 3

Effects of BPA on levels of O2-, H2O2, •OH and MDA content of leaves after ten days of exposure. The error bars indicate standard deviation (n=3). The asterisk (*) indicates significant differences ($p<0.05$) with compared to single species control (sp(A), sp(B), sp(C)), respectively.

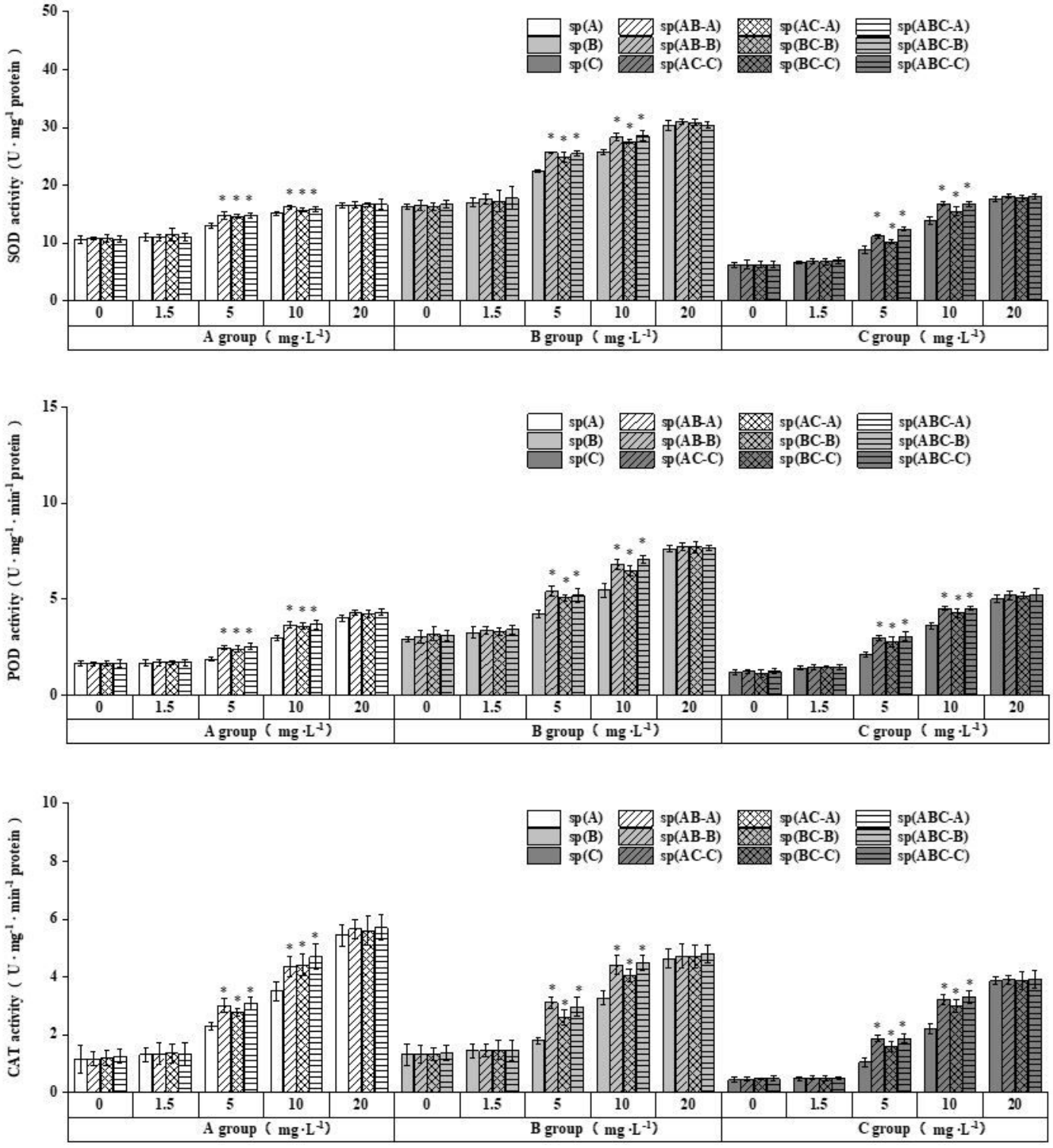

Figure 4

Effects of BPA on SOD, POD, and CAT activities of leaves after ten days of exposure. The error bars indicate standard deviation (n=3). The asterisk (*) indicates significant differences ($p<0.05$) with compared to single species control (sp(A), sp(B), sp(C)), respectively.

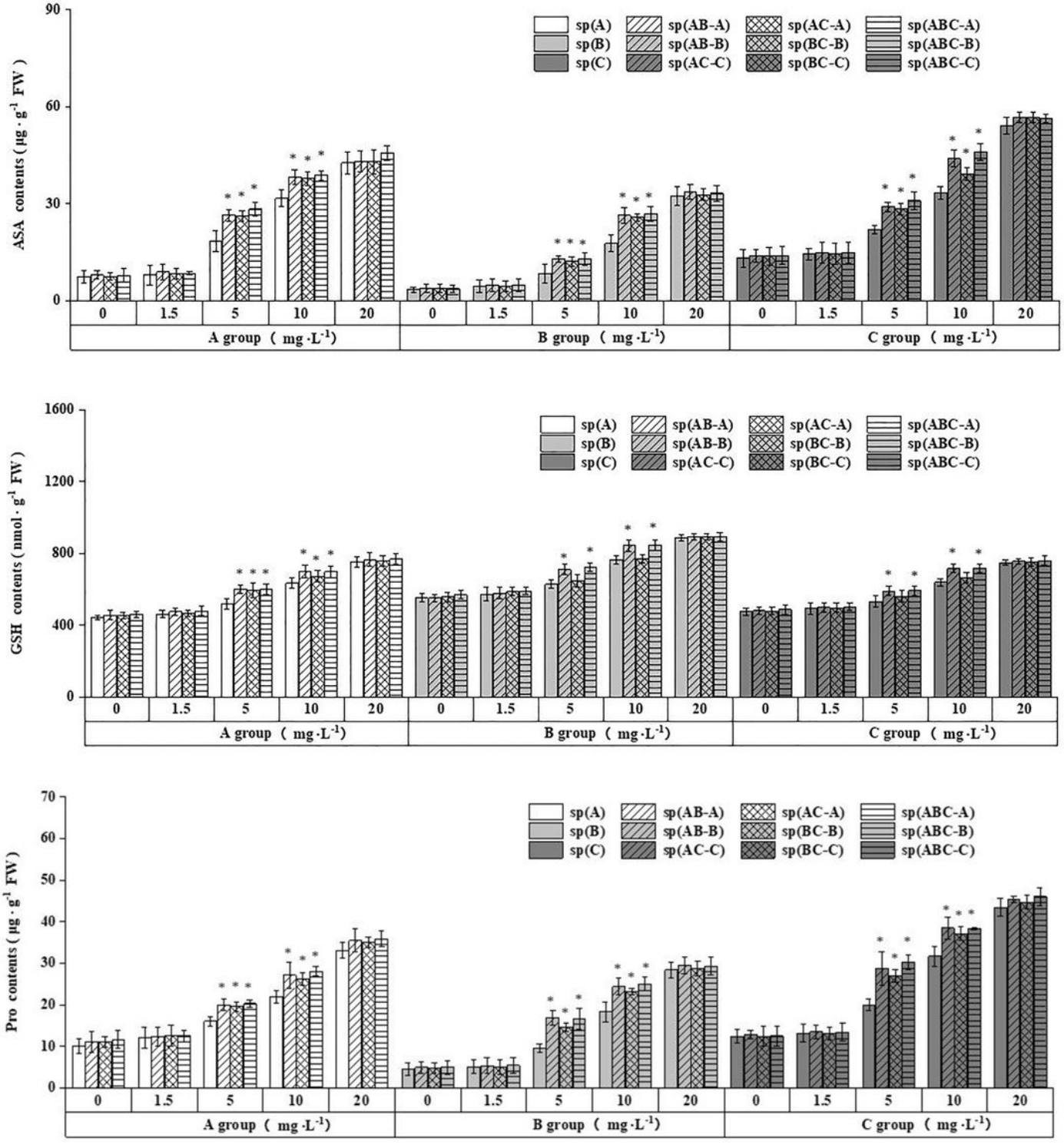

Figure 5

Effects of BPA on the ASA, GSH and Pro contents of leaves after ten days of exposure. The error bars indicate standard deviation (n=3). The asterisk (*) indicates significant differences ($p<0.05$) with compared to single species control (sp(A), sp(B), sp(C)), respectively.

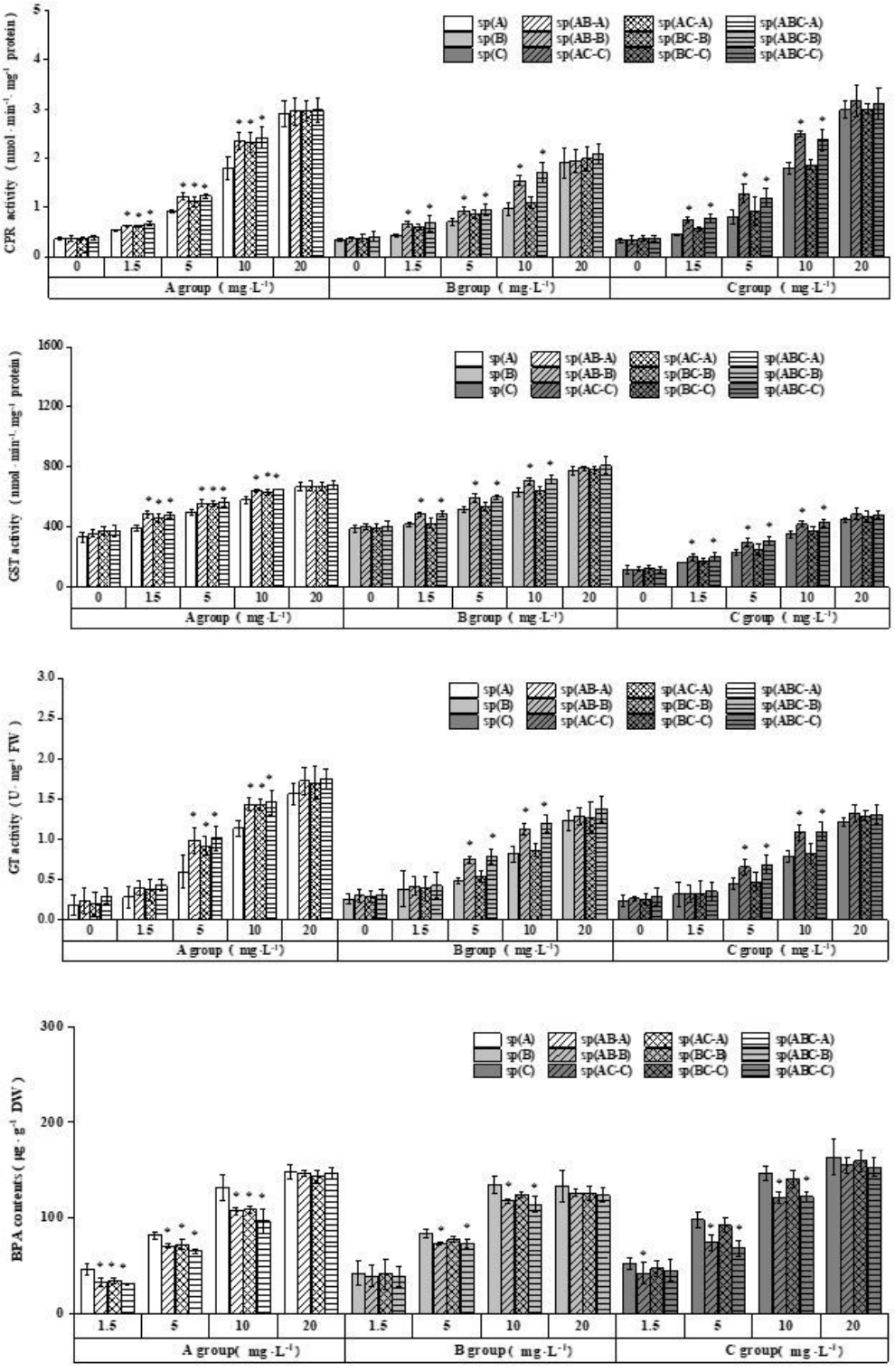

Figure 6

Effects of BPA on the CPR, GST, GT activities and BPA contents of leaves after ten days of exposure. The error bars indicate standard deviation (n=3). The asterisk (*) indicates significant differences ($p<0.05$) with compared to single species control (sp(A), sp(B), sp(C)), respectively.

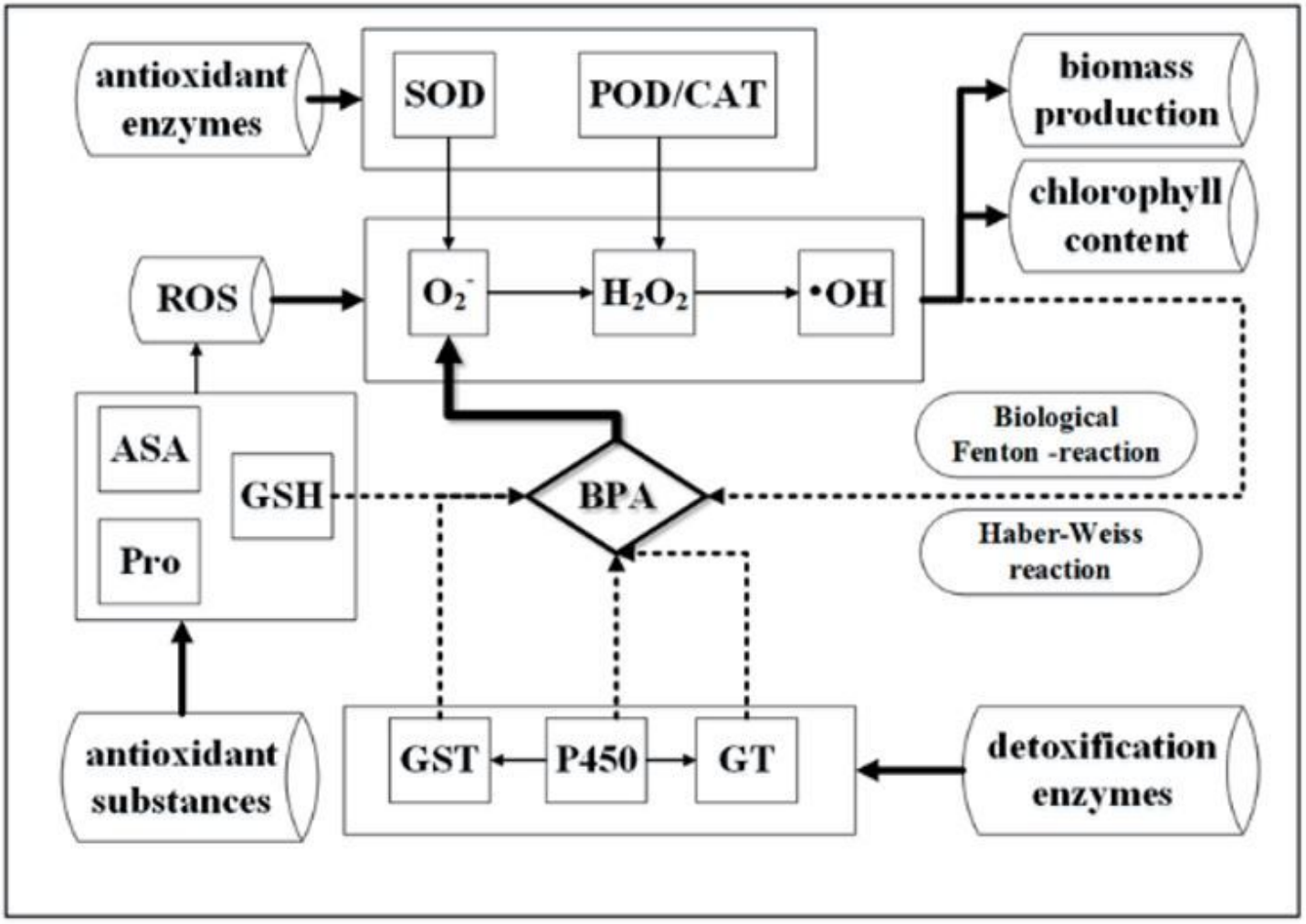

Figure 7

A proposed model depicting of the plant species compositions alleviate toxicological effects of BPA.