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RESEARCH ARTICLE

Evaluation of an innovative approach based on prototype engineered wetland to control and manage boron (B) mine effluent pollution

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Abstract A major environmental problem associated with boron (B) mining in many parts of the world is B pollution, which can become a point source of B mine effluent pollution to aquatic habitats. In this study, a cost-effective, environment-friendly, and sustainable prototype engineered wetland was evaluated and tested to prevent B mine effluent from spilling into adjoining waterways in the largest B reserve in the world. According to the results, average B concentrations in mine effluent significantly decreased from 17.5 to 5.7 mg l^{-1} after passing through the prototype with a hydraulic retention time of 14 days. The results of the present experiment, in which different doses of B had been introduced into the prototype, also demonstrated that *Typha latifolia* (selected as donor species in the prototype) showed a good resistance to alterations against B mine effluent loading rates. Moreover, we found that soil enzymes activities gradually decreased with increasing B dosages during the experiment. Boron mass balance model further showed that 60 % of total B was stored in the filtration media, and only 7 % of B was removed by plant uptake. Consequently, we suggested that application of the prototype in the vicinity of mining site may potentially become an innovative model and integral part of the overall landscape plan of B mine reserve areas worldwide.

Keywords Boron mine pollution · Engineered wetlands · Prototype approach · Secondary benefits

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Introduction

Boron (B) mine reserves are strategic regions worldwide due to their supply of unique B compounds and minerals that are necessary to human society (Özdemir and Kıpçak 2010; Thakur et al. 2013). Boron reserves on the earth's crust are located in Turkey, USA, Russia, China, Chile, Argentina, and Peru (Türker et al. 2013). Mining pressure on the B mine reserve areas is very high, and future scenarios suggest that it may reach maximum levels in the coming years because B is so widely used for commercial and industrial purposes (Taştan et al. 2012; Türker et al. 2013; Ramilá et al. 2015). Therefore, the problem of B mining contamination in countries with natural deposits has recently become a critical issue (Wolska and Bryjak 2013). A major environmental problem associated with B mining in many parts of the world, especially in Western Anatolia (Turkey) and California (USA), is related to uncontrolled effluents (Okay et al. 1985; Stiles et al. 2011; Türker et al. 2013, 2014a). During B mining operation, B-rich effluent can leak and diffuse into the receiving water body due to erosion and rainfall. In this respect, B pollution generated and accumulated over the whole water body is transformed into a point source of boron mine effluent pollution upon entry into the aquatic habitats (Stiles et al. 2011; Böcük et al. 2013; Wurl et al. 2014). Therefore, aquatic habitats around B mine reserve sites can suffer from moderate to high levels of boron contamination which may degrade the ecological balance of the local ecosystem (Böcük et al. 2013; Türker et al. 2013; Ramilá et al. 2015). It is clearly necessary to evaluate a new holistic and sustainable strategy for the prevention and control of B mine effluent in order to protect water resource.

Engineered wetlands (EWs) or constructed wetlands (CWs) are an eco-technological approach to treat and control point or non-point source pollution, and thus they are now widely used as an alternative and effective method to reduce environmental pollution all over the world (Chen et al. 2014). Hence, the reports of wastewater purification by EWs have dramatically increased in the scientific literature (Vymazal 2013). However, information about the use of an EW for the prevention and control of B pollution directly in mining effluents outlets in B mine ecological environments is limited. Although some pioneering research reported that EWs could also be used as potential treatment options due to their ability to remove B from wastewater (Ye et al. 2003; Allende et al. 2012; Türker et al. 2013, 2014a; Allende et al. 2014), these researches have various limitations in their practices: (1) information about using an EW to optimize sustainable land management strategy in actual mine reserve areas is still insufficient, (2) there is lack of knowledge in monitoring physiological plants in wetland matrix responses to B, (3) there are no evidences related to the effect of B on soil biochemistry in EWs or CWs, and (4) the importance of potential secondary benefits such as those obtained from the EWs is not sufficiently discussed, and most researches have not focused on facilitating and optimizing EW design in terms of the innovative approach.

Therefore, the main objectives of this study were (1) to evaluate and test a cost-effective, environment-friendly, and sustainable prototype EW to control and prevent B mine effluent pollution under field conditions in the largest B reserve in the world. The goal is to position the prototype within the mine area as to intercept and filter B mine effluent before it leaks out into surface water, (2) to monitor *Typha latifolia* (as a donor plant selected in this study) growth and its physiological responses against B at the different mine effluent loading rates, (3) to investigate the effects of B on soil enzyme activities such as dehydrogenase, protease, and urease in the prototype, and (4) to assess potential secondary benefits of the prototype as an innovative model for the overall landscape plan of B mine reserve areas all over the world.

Material and methods

Description of research area

The experiment was performed from March to November 2014 at the largest Borax mine reserve area (39° 17′ N, 30° 30′ E) in the world (Kırka country—Eskişehir in Turkey) under natural climatic conditions. This area is a leading mine region in Turkey with extensive boron mining activities. Borax pentahydrate is the main commercial product mined from this reserve area. During B mining and extraction in this reserve, large amounts of boron ore are excavated because the ore contains mostly clay and the B amount in ore is between only 20 and 28 % (Böcük et al. 2013). Non-target B-rich waste including clayey materials in the B ore is removed after the excavation process. A large area (approximately 5 km ^{2}) within the mining area was selected as a reservoir or re-stripping area in order to store non-target B-rich waste. However, some portions of that area with the stored waste floods between 0.2 and 1 m during the rainy period, especially in early spring and autumn. At such times, the B-rich waste can leak from the re-stripping area into the receiving water body, and thus the aquatic ecosystems around the mine sites can be direct or indirect recipients of B effluent. A suitable research site in the re-stripping area of Kırka B Mine Reserve was selected to test the prototype under actual environmental conditions.

Prototype design and cultivation period

The newest literature reports provide some information about an attractive experimental wetland design parameter for B removal (Türker et al. 2014b; Allende et al. 2014). According to these former experiments, the highest B removal efficiency was achieved by combining a mono-culture planting design and sub-surface flow regime in EWs, so the monoculture design guide was applied in the present study (Türker et al. 2013, 2014b). Hence, two pilot-scale wetland systems with monoculture design parameter were constructed, prepared, and located outdoors at the research site. One of the wetlands was selected as treatment prototype, while the other was used as an unplanted control. Each prototype was 2 m in length, 1 m in width, and 0.6 m in depth (water depth is 0.4 m) with a surface area of 2 m^2, and employed gravity feed using 1.5° slope (Fig. 1).

Various researchers recommend the use of organic-based media to improve retained B in EWs (Ye et al. 2003; Allende et al. 2012, 2014). Moreover, Ye et al. (2003) emphasized the use of calcareous sand to contribute to the removal of B due to co-precipitation of B with calcium. Therefore, the prototypes were filled with a mixture of organic-based peat (pH 4.6) and calcareous sand (45 % $CaCO_3$) to a depth of 0.5 m. Approximately 5 cm of gravel was also placed on the mixture of organic-based peat and calcareous sand. The approximate percentages of the three filling materials in mixture within the prototypes units were 40 % organic peat, 40 % calcareous sand, and 20 % gravel. Coarse gravel was added in the inflow and outflow section, and a plastic pipe was installed at the center of each prototype to measure water depth. The mixture which was prepared as filtration media was analyzed (in terms of B amount) and washed prior to being inserted into the prototypes (the boron content of filtration mixture was found to be negligible). Detailed information about the schematic demonstration of prototypes is given Fig. 1.

The use of local native species to plant EWs can improve pollutant removal process (Vymazal and Kröpfelová 2008).

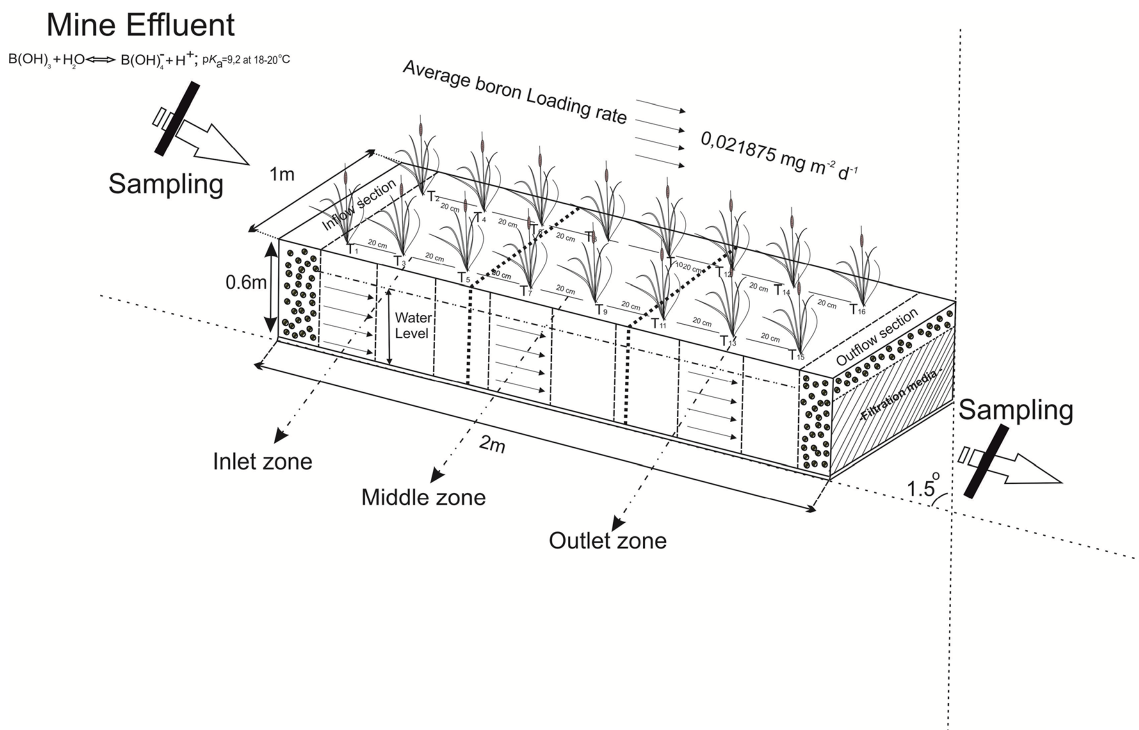

Fig. 1 Schematic demonstration of the prototype engineered wetland (PEW)

Furthermore, previous studies indicate that particularly, *T. latifolia* L. (cattail) can adapt better to an organic-based media in EWs compared to the other macrophytes due to the highly aggressive and competitive capability of *T. latifolia* colonizing in EWs under natural conditions (Türker et al. 2013, 2014a). Therefore, the prototype was vegetated with *T. latifolia* which can grow in the B-rich aquatic environments surrounding the B reserve. Rhizomes of *T. latifolia* were collected from natural wetlands surrounding Kırka B Reserve, and the rhizomes were immediately transplanted in prototype at a plant density of about 8 rhizomes/m^2. Later, the rhizomes were numbered and recorded according to their location in the prototype.

Prior to the start of the experiment, the prototypes were fed by secondary-treated wastewater for 90 days in order to support the plant growth and establish microorganisms. The secondary-treated wastewater was collected from the settling pond of the neighborhood.

Boron dosage and wetland operation

After the cultivation period of 3 months, the prototype and unplanted control were fully established for the treatment facility under the field conditions. Mine effluent, which was obtained from a pond surrounding the re-stripping area in the B mine reserve site, was used for the present experiment for the application of the prototype under the field conditions. The mine effluent was modified by adding secondary-treated wastewater in order to adjust the B level to appropriate values for the study because B concentration of the mine effluent in the pond changed seasonally, ranging between 6 and 40 mg l^{-1} in the water throughout the year. This is also the reason why and how we determined the range of B concentration used in this study. Element concentrations in the mine effluent obtained from the pond and in secondary-treated wastewater before the experiment period are shown in Table 1.

The prototype and unplanted control were filled with test solution from a storage tank containing B mine effluent, and

Table 1 Element concentrations in the mine effluent and in the secondary-treated wastewater used in the present experiment before the study period

Parameter	Unit	Mine effluent	Secondary-treated wastewater
Boron (B)	mg l^{-1}	40.8	2.49
Silver (Ag)	mg l^{-1}	0.009	0.0075
Cadmium (Cd)	mg l^{-1}	<d.l.	<d.l.
Calcium (Ca)	mg l^{-1}	54.8	102.8
Chromium (Cr)	mg l^{-1}	<d.l.	<d.l.
Copper (Cu)	mg l^{-1}	<d.l.	<d.l.
Iron (Fe)	mg l^{-1}	<d.l.	<d.l.
Sodium (Na)	mg l^{-1}	81.62	37.44
Nickel (Ni)	mg l^{-1}	<d.l.	<d.l.
Lead (Pb)	mg l^{-1}	0.0086	0.0433
Zinc (Zn)	mg l^{-1}	<d.l.	<d.l.
Manganese (Mn)	mg l^{-1}	0.0183	0.0462
Potassium (K)	mg l^{-1}	17.50	7.934

<*d.l.* below the detection limits

the effluent was fed into the inflow zone. The effluent was pumped through pipes into each system, and each feeding cycle took 1 h. During the research period, the prototype and unplanted control were manually drained with a valve at the bottom of each system every 2 weeks and then were refilled with fresh mine effluent containing different B concentrations. The systems operated under the same hydraulic loading rate (HLR) of 0.021875 mg m^{-2} day^{-1} and the hydraulic retention time of systems was to set to 14 days. These types of hydraulic regimes have a high potential for the removal of contaminants such as B from wastewater (Türker et al. 2013). The prototypes operated continuously for a period 154 days until vegetation showed wilting at the beginning of November 2014.

Chemical analysis and sampling of water, plants, and media

Water sampling and analysis

Water samples were taken every 2 weeks, at intervals based on the hydraulic retention time of the prototypes according to Türker et al. (2013, 2014a). Samples for the chemical analyses were collected from inflow and outflow of each prototype. Concurrent with sampling, pH, electrical conductivity (EC), temperature, dissolved oxygen (DO), and redox potential were measured with a HACH HQ40D multi-parameter meter in the field. For the elemental analyses, the samples were filtered through Whatman cellulose filter paper and acidified to pH <2 with nitric acid (65 %). The concentration of B, Ca, K, and Na were determined with a high-resolution continuum source atomic absorption spectrometer (Analytikjena ContrAA 700). Samples for nitrate and nitrite were measured with a colorimetric method using a colorimeter (HACH DR/890) immediately after each sampling period. Because Ag, Cd, Cr, Cu, Fe, Ni, Pb, Zn, and Mn concentrations in the mine effluent are below the detection limits or drinking-water standards before the study, the concentration of these elements in the mine effluent are not monitored during the experiment period (Table 1).

Plant monitoring, harvesting, analysis, and biomass production

The height and shoot numbers of *T. latifolia* were measured every 2 weeks, and any instances of plant disease or insect attack were recorded. Plant height was calculated as the average height of individuals in the prototype, and plant density was determined from the average shoot numbers of individual species in the prototype. Chlorophyll content of plants in the prototype was also measured in the experiment. For the analysis, fresh tissues from mature leaves of plants were collected weekly from the prototype, and chlorophyll *a* and *b* content were measured according to Wellburn (1994).

At the end of the study, all the plant biomass both aboveground and belowground was harvested from the prototype in order to calculate dry biomass and chemical composition of plants, and also to determine the plant biomass production which can be converted to potential biogas. Dry biomasses of the aboveground and belowground parts were calculated by drying the harvested biomass in an oven at 65 °C for 48 h. After calculating, individuals were separated into leaves, stems, and roots, and all the material were powdered and digested by $HClO_4/HNO_3$ acid at 1:3 proportions in a microwave digestion unit. The concentration of B, Ca, K, and Na were determined with atomic absorption spectrometer. Moreover, ash content of plants was determined according to Cria et al. (2005).

The convertible plant biomass to biogases was calculated theoretically according to Kaltwasser (1980) and Buswell (1952) based on harvestable biomass after the study. Moreover, we calculated the potential methane production depending on biomass production with the Buswell equation as:

$$C_xH_yO_z + \left(x - \frac{y}{4} - \frac{z}{2}\right)H_2O \rightarrow \left(\frac{x}{2} - \frac{y}{8} + \frac{z}{4}\right)CO_2 + \left(\frac{x}{2} + \frac{y}{8} - \frac{z}{4}\right)CH_4 \quad (1)$$

In this basic equation, C: carbon, H: hydrogen, O: oxygen, and x, y, z: number of atoms of elements.

Filtration media analysis

The media samples from the prototype and the unplanted control were collected homogenously from the inlet zone, middle zone, and outlet zone at the end of the experiments. The samples were collected using a 4-cm-diameter PVC corer. The concentrations of B, Ca, K, and Na were determined using the atomic absorption spectrometry method. The extractable B (plant-available B) in the media was measured with carminic acid method, N by Kjeldahl method, and soil structure with Bouyoucos method (Türker et al. 2013, 2014a). Moreover, rhizosphere samples from the prototype and sediment samples from the unplanted control system were also collected every 2 weeks using hand-shaking method by avoiding edge effects between supporting media and rhizosphere (Zhang et al. 2010a). The collected the field-moist media samples were sieved and then stored in a refrigerator at 4 °C prior to analysis of enzyme activity within 1 week. The dehydrogenase, protease, urease, and enzyme activities in filtration media of the prototypes were determined according to Kong et al. (2009).

Calculations and B mass balance model

Boron mine effluent loading rate to the prototype and the unplanted control was calculated as described by Debing et al. (2009):

$$\text{Loading rate}\left(\text{mg m}^{-2}\text{day}^{-1}\right) = \left[(C_i \times V_i)\big/(S \times I)\right] \quad (2)$$

where C_i refers to the average B concentration inflow (i.e., raw water flowing into the system), V_i corresponds to standing mine effluent volume in the prototype and the unplanted control, S is the total unit area of the prototype, and I is the time between water drainage and refilling.

Boron removal efficiency (%) from mine effluent passing though the prototype and the unplanted control was calculated as follows:

$$\text{B removal efficiencies } (\%) = \left[(C_iV_i - C_eV_e)\big/C_iV_i\right] \times 100 \quad (3)$$

where C_i and C_e are the average B concentrations of inflow and outflow (mg l^{-1}), and V_i and V_e are the volumes of the outflow and the inflow collected and dosed in the prototype and the unplanted control.

The mass removal rate (MRT) was calculated using the equation evaluated by Wu et al. (2011):

$$\text{Mass removal rate } \left(\text{mg m}^{-2}\text{day}^{-1}\right) = \left[(C_i \times V_i - C_e \times V_e)\big/\text{unit surface area}\big/\text{HRT}\right] \quad (4)$$

The unit area of the prototype and the unplanted control was 2 m^2 and hydraulic retention time (HRT) was 14 days as described above.

A simple conceptual model was designed in order to investigate the contribution of various removal pathways in removing B in the prototype.

The mass balance model is obtained and is shown below:

$$B_{\text{input}} = B_{\text{output}} \quad (5)$$

where B_{input} refers to the total amount of B (mg) added in the prototype during the research period and B_{output} is the total amount of B (mg) collected from the prototype in the research period.

$$B_{\text{input}} = W_{\text{flowing}} \times B_{\text{inflow}} \quad (6)$$

$$B_{\text{inflow}} = C_i \times V_i \quad (7)$$

$W_{flowing}$ is the total amount of water flowing through the prototype during the experiment (m^3) (for 154 days) and B_{inflow} corresponds to the B concentrations in inflow. C_i is the average inflow B concentrations (mg l^{-1}), and V_i is the volume of the inflow in liters.

$$B_{\text{output}} = B_{\text{outflow}} + B_{\text{plant}} + B_{\text{media}} + B_{\text{undetermined}} \quad (8)$$

$$B_{\text{outflow}} = C_e \times V_e \quad (9)$$

where $B_{outflow}$ is the average B concentrations in outflow. C_e is the average outflow B concentrations (mg l^{-1}), and V_e is the volume of the outflow in liters.

$$B_{\text{Plant}} = (T_{end} \times B_{end})\big/1000 \quad (10)$$

where B_{Plant} and T_{end} are B in plants and total amount of plant biomass at the end of the experiment (g), respectively. B_{end} corresponds to concentrations of B in plants end of the study (mg/g).

$$B_{\text{media}} = (W_{\text{media}} \times T_{\text{media}})\big/1000 \quad (11)$$

B_{media} is the B in media, W_{media} refers to the total amount of media (weight in grams), and T_{media} is the total B in media at the end of the study (mg/g).

$$B_{\text{undetermined}} = B_{\text{input}} - B_{\text{outflow}} - B_{\text{plant}} - B_{\text{media}} \quad (12)$$

where $B_{undetermined}$ is the undetermined B amount during the research period.

Statistical analysis

In this study, statistical relations between the concentrations of B and pH, EC, and temperature value in inflow and outflow water samples for the experimental prototype and unplanted control were determined using one-way ANOVA test. Correlations between B removal efficiency and physicochemical parameter of wastewater in the prototypes were analyzed by Pearson's correlation coefficient. PCA statistical analysis was used to ascertain whether the concentrations of B and Ca, K, Na, and N in *T. latifolia* were related to different parts of treatment area in the prototype. The data in the experiment were checked for normality and homogeneity of variance (using the Shapiro-Wilks test) with significance set at 0.05 before performing ANOVA. Statistical confidence was set at $p < 0.05$, and all statistical calculations were implemented using SPSS version 19.0 of the Statistical Software Package.

Results and discussions

Filtering performance of the prototype

Figure 2 illustrates inflow and outflow properties, B removal efficiency, and mass removal rate in both the prototype and the

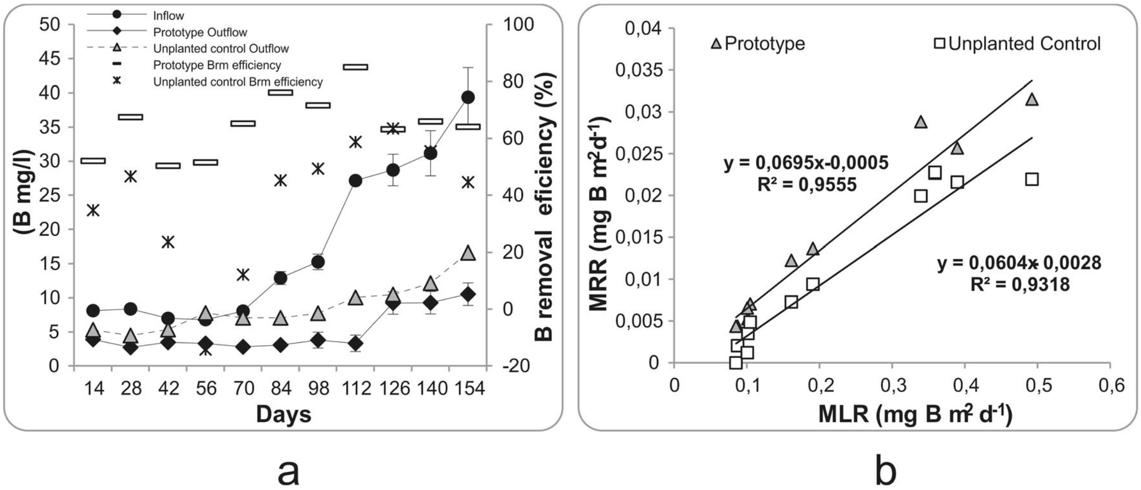

Fig. 2 Inflow and outflow properties, boron removal (*Brm*) efficiency and mass removal rate, in both prototype and unplanted control over the entire experiment period

unplanted control over the entire experiment period. As seen in Fig. 2a, B concentrations in the inflow gradually increased during the study period reaching an average value of 17.5 mg l^{-1} and B concentrations in inflow decreased from 17.5 to 5.03 mg l^{-1} after passing through the prototype ($p < 0.05$). This result demonstrates that the prototype is capable of removing B from the mine effluent, and thus the prototype could be a reasonable bio-filter option to control B pollution directly from mining effluent outlets in B mine reserve areas all over the world. This is in agreement with various authors in the literature who reported that EWs can treat B from various types of wastewater (Ye et al. 2003; Gross et al. 2007; Allende et al. 2012, 2014; Türker et al. 2013, 2014a).

On the other hand, B removal efficiency of prototype fluctuated greatly in the range of 50–84 % over the study period, and B removal performance of the prototype gradually increased with increasing B concentration in the mine effluent. Accordingly, B concentration in outflow samples of prototype was stable below 5 mg l^{-1} during the first 112 days, and the highest B removal performance was achieved at the end of the 112th day. However, B removal performance of the prototype suddenly decreased after the 112th day when inflow B concentrations exceeded 27 mg l^{-1}. This sudden decrease in the present study could result from the fact that prototype components including supporting media and plants started to saturate B when inflow B concentrations reached 27 mg l^{-1}, and thus B removal performance decreased due to the overload of filtering capacity of the prototype. The data from the previous study also support these phenomenon, and it can be seen that the B removal efficiency was initially high but then decreased when adsorption capacity of filtration media and plants were used up gradually (Ye et al. 2003; Gross et al. 2007; Allende et al. 2012, 2014). Therefore, in order to optimize or stabilized B removal efficiency of the prototype during B purification process, our results suggest that inflow B concentration should not be exceeding 27 mg l^{-1}.

Applied B loading rates and corresponding mass removal rates of B, together with the coefficient of determination (R^2) values obtained by regression analysis, for both the prototype and the unplanted control are also represented in Fig. 2b. It can be seen that the B mass removal rates of the prototype were slightly higher than that of the unplanted control throughout the investigation. This slightly higher mass removal rate of the prototype may be as a result of improved filtration through *T. latifolia*'s rooting biomass in the prototype and/or the accumulation of B by *T. latifolia* from the mine effluent. Moreover, we found that a good linear correlation between the incoming B mass loads and B mass removal rates were observed for the mine effluent in both the prototype and the unplanted control. The boron mass removal rates of the prototype (0.004–0.031 mg m^{-2} day^{-1}) and the unplanted control (0–0.022 mg m^{-2} day^{-1}) showed strong correlation with incoming loads, with R^2 values of 0.9555 and 0.9318, respectively. Therefore, the differences in the correlation of incoming B mass loads at varying mass removal rates of the prototype and the unplanted control were an indication that higher B removal rate could be achieved when the inflow B concentration was high as reported before by Türker et al. (2013) and Türker et al. (2014a).

pH value of water also can affect B removal performance of EWs by changing wetland dynamics, especially with respect to B in EWs. As previously reported by Oertli and Grgurevic (1975), availability of B for plants and media increases near neutral pH levels, and thus the adsorption of B in filtration media and plant tissues increases with near neutral pH levels. As shown in Fig. 3, pH value showed a slight fluctuation both in the prototype and the unplanted control during the experiment period, and the pH value of the prototype was significantly lower than the unplanted control and closer to pH 7.0

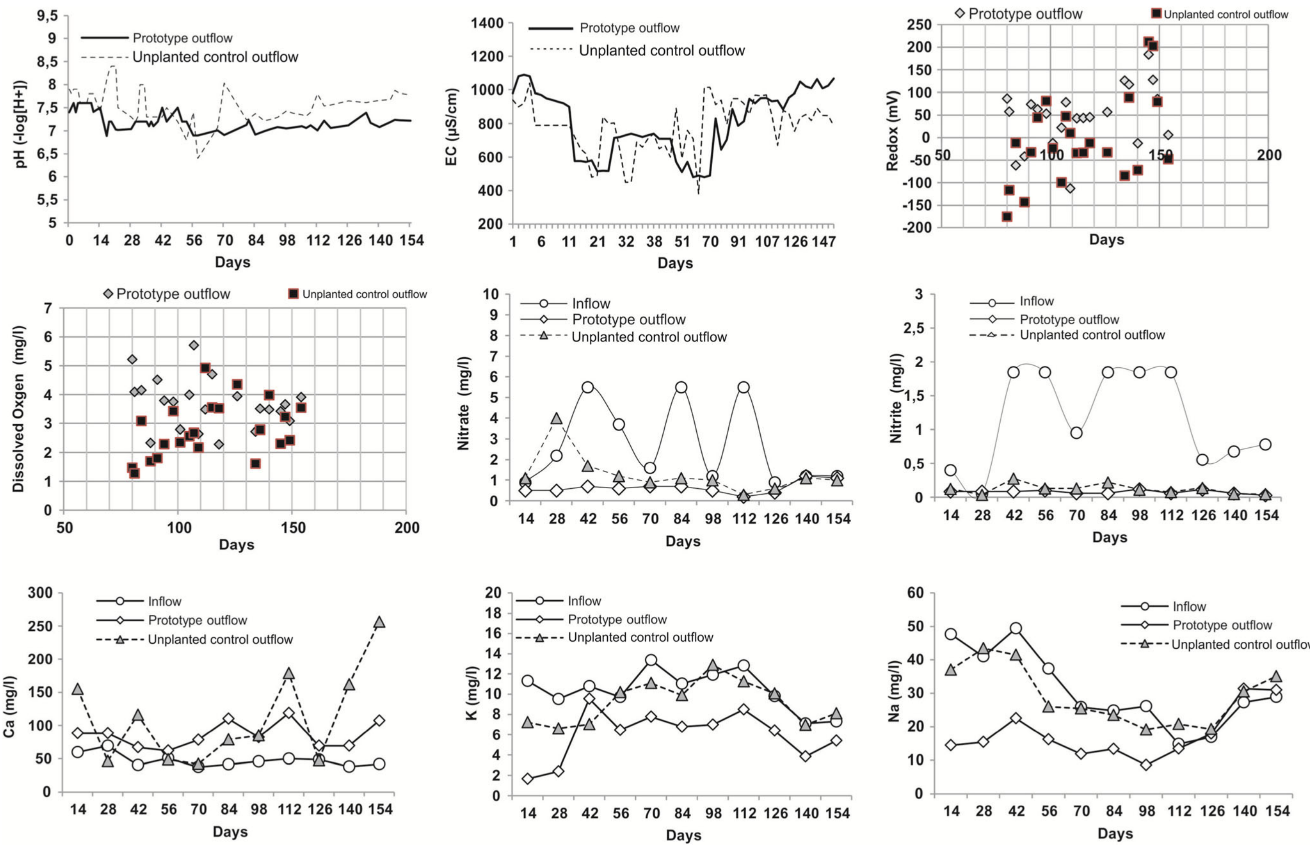

Fig. 3 Physicochemical parameter and element concentrations of inflow and outflow both in the prototype and unplanted control during the study

($p < 0.05$). This result demonstrated that B is more available in the prototype as compared to the unplanted control and suggested that plants in the prototype may play an important role in the removal of B from the mine effluent. Therefore, use of plants in EWs could maximize B removal efficiency. Moreover, differences in pH value in the planted prototype would mean that the development of vegetation and plant roots over time may have affected pH value through release of acidic exudates such as allelopathic chemicals from plant roots into the water and root respiration of plants, so they could have also contributed to B removal dynamics in the planted prototype (Türker et al. 2014a).

One-way ANOVA indicated that EC values of outflow were not significantly lower than those of the inflow into the prototype ($p > 0.05$). Moreover, there is no significant difference determined between the prototype and unplanted control (Fig. 3) ($p > 0.05$). This result indicated that anionic and cationic ions were not significantly reduced in either the prototype or the unplanted control during B purification process, suggesting that EC removal mechanism of the prototype is related to mostly physical processes such as sedimentation, and the effect of plant filtration on EC removal might not be important (Leto et al. 2013).

Figure 3 shows that no discernible temporal trends were observed in both redox potential and dissolved oxygen level for the prototype and the unplanted control throughout the experiment. Moreover, we found that there are no significant correlations among redox potential ($r = +0.212$; $p > 0.05$) and dissolved oxygen ($r = +0.028$; $p > 0.05$) with B removal efficiency. Nevertheless, it can be concluded that redox potential in the prototype reached mostly positive values once the experiment started, and the redox values changed between +100 and −100 mV until the 130th treatment day. However, the redox values in the prototype quickly increased from +100 to +200 mV after the 130th treatment day and remained close to +150 mV. These results indicated that oxidized iron in the prototype was relatively stable during the experiment period and the oxidation of ferrous ions may be maintained by the iron-depositing bacteria which are associated with the development of a redox gradient in the prototype matrix. Moreover, the precipitation of iron hydroxides in the rhizosphere related to the activities of iron-depositing bacteria probably leads to an iron plaque formation, and this plaque promotes B binding affinity in the organic-based media, potentially being permanently immobilized in the prototype as reported by Vymazal and Kröpfelová (2008) for other trace elements. On the other hand, it can be seen in Fig. 3 that the redox value of the prototype is mostly higher than the redox values in the unplanted control during the experiment period. This suggested that *T. latifolia* in the prototype affected the redox status in the rhizosphere due to its ability to release oxygen with roots, and thus *T. latifolia* can influence B mobility in the prototype matrix. Hence, it can be hypothesized that increasing oxygen release by *T. latifolia* at the rhizosphere into the prototype may have resulted in more Fe precipitation as iron plaque compared to the unplanted control, and this may have led to co-precipitation associated with the presence of *T. latifolia* and the action of the iron-depositing bacteria in the prototype (Vymazal and Kröpfelová 2008). Theoretically, B removal dynamics could be related to the aerobic oxidation pathways in the rhizosphere, and increased oxygen release at the rhizosphere can have more positive benefits improving B removal in the planted prototypes. However, further researches are needed to understand the relationship between aerobic oxidation pathways or redox conditions of rhizosphere and B removal efficiency in wetland which are designed for remediation.

In the treatment period, nitrite and nitrate concentrations in the prototype outflow were significantly lower than those in the inflow ($p < 0.05$) (Fig. 3). However, we did not find any statistical differences between the planted prototype and the unplanted control in terms of nitrite and nitrate removal ($p > 0.05$). Therefore, it can be concluded that nitrite and nitrate together with B were efficiently reduced by the prototype, and nitrogen removal mechanism in EW is related to mostly biological and physical processes such as nitrification-denitrification (Vymazal 2013). On the other hand, no discernible removal trends were observed for Ca, K, and Na in the prototype and the unplanted control throughout the experimental period. Moreover, the Ca concentrations in the inflow were mostly lower than the prototype and the unplanted control outflow. The reasonable explanations for this situation might be that the filtration material in the prototype was saturated with Ca during the study because of using calcareous sand in the filtration media, and that is why we measured higher Ca concentration in outflow sample. K and Na concentrations in the outflow samples of the prototype were relatively lower than those in the inflow ($p > 0.05$) (Fig. 3).

Plant growth, monitoring, and boron uptake

The selection of plant species is an important issue in the EWs and related to their lifespan (Vymazal and Kröpfelová 2008). In the present experiment, the survival rate of *T. latifolia* in the prototype after the culture period was found to be higher than 75 % according to Türker et al. (2013), suggesting that *T. latifolia* was well adapted to climatic as well as operational conditions of the research area and thus *T. latifolia* can be suitable species in the prototype.

The plant height and number of the shoots in the prototype were monitored during the prototype operation, and the results are shown in Fig. 4a. The monitoring period was divided into three stages based on B mine effluent loading rates in order to observe the alterations in plant growth due to B mine effluent loading rates applied to the prototype: the first feeding period from day 0 to 51 (BLR 0.0084–0.0104 mg m^{-2} day^{-1}), the second feeding period from day 52 to 107 (BLR 0.01–0.0339 mg m^{-2} day^{-1}), and the third feeding period from day 108 to 154 (BLR 0.0358–0.0492 mg m^{-2} day^{-1}) (Fig. 4). After the first application of effluent, plant height gradually increased and reached almost 100 cm. At the same time, a similar trend also was found for the number of shoots in the prototype during the first feeding period. At the second feeding period, *T. latifolia* reached its maximum height and then remained constant until the 112th day. However, the number of shoots in the prototype also continued an increasing trend during the second feeding period and reached about 80 cm. A slight decreasing trend associated with the decay of dry leaves was determined for the height of *T. latifolia* in the third feeding period, whereas the numbers of shoots in the prototype slightly increased and then remained constant until the end of the experiment. Furthermore, *T. latifolia* in the prototype grew well and did not show any signs of B toxicity symptoms including necrosis and chlorosis or another nutrient deficiency during the first, second, and third feeding period. These results indicate that *T. latifolia* in the prototype showed a good resistance to alterations in B mine effluent loading rates, and thus higher growth rate and proliferous germination level were achieved for *T. latifolia* in the prototype during the experimental period. Therefore, we recommend that *T. latifolia* is a well-suited option against potential toxic effects of B mine effluents and their variability in the prototype.

The chlorophyll content in the plants was determined to assess B mine effluent toxicity during the experiment period. The levels of the chlorophyll pigments *a*, *b*, as well as total pigment concentrations ($a + b$) for *T. latifolia* in the prototype are shown in Fig. 4b. The chlorophyll pigment concentrations of *T. latifolia* in the first feeding period were higher than in the second and third feeding periods. It may be explained by several causes such as hostile climatic pressure in the research area and operational origin associated with excess B concentrations in the mine effluent. On the other hand, higher chlorophyll content, especially in the first and second feeding periods, may indicate the apparently higher resistance of *T. latifolia* to the application of B mine effluent. Therefore, further investigations are required to assess the detoxification pathways of *T. latifolia* against B in the wastewater.

Contents of B and some other elements in *T. latifolia* tissues according to plant number and location in the prototype are shown in Table 2. *T. latifolia* that were located in the outlet zone acquired more B than those toward the inlet and middle. This result indicated that the effective uptake facility of B occurred within the outlet zone, and thus plants near the outlet zone play an important role in accumulating B from mine effluent. The higher uptake by plants near the outlet zone of the prototype may be explained by the higher bioavailability

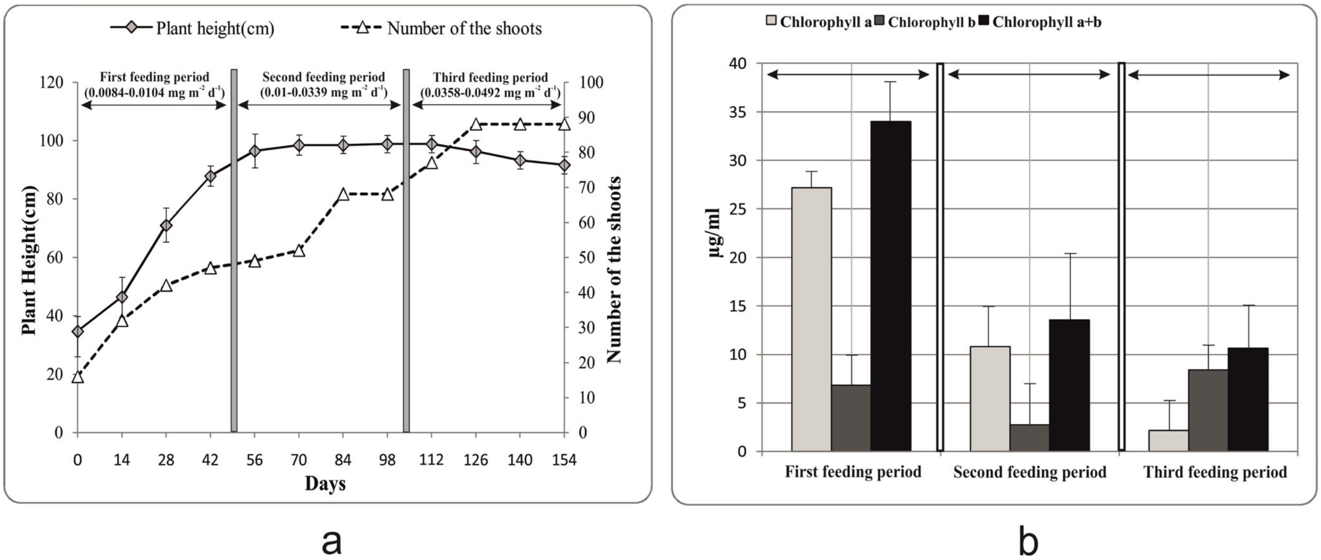

Fig. 4 Plant height and number of the shoots in prototype (**a**) and the level of the chlorophyll pigments *a* and *b*, as well as total pigment concentration (*a* + *b*) for *T. latifolia* in the prototype (**b**). *Error bars* indicate standard deviation

of B related to $CaCO_3$ and Ca content in the media of the prototype (Ye et al. 2003). Accordingly, higher concentrations of B as a form of boric acid are dissolved into the outlet zone in the prototype, and thus more B is available for plant uptake in this zone. That is why B is accumulated from aqueous environment by plants mainly as a form of boric acid and stored in their tissues. The presence of high concentrations of boric acid in the outlet zone most likely leads to a significant effect on B uptake by plants. Therefore, the effects of the location on B accumulation by plants should be taken into consideration when designing such prototypes. Moreover, we believe that for ions such as B to be accumulated by plants in the prototype, they should exist in plant-available concentrations in supporting media to maximize the accumulation capacity of the plants and contribute to the success of prototype (Ye et al. 2003; Allende et al. 2014).

Our results also demonstrate that leaves of *T. latifolia* have greater B accumulation compared to stems and roots, and B content in leaves were nearly two times higher than that of the stems and roots. These results showed that a significant translocation occurred in *T. latifolia* and B in mine effluent is transported to leaves from roots, which is consistent with the previous study reported by Türker et al. (2014a). Therefore, harvesting might be a viable B removal option for the prototype among current management practices. In such cases, a considerable amount of accumulated B can be removed from mine effluent by frequent harvesting of the plants. Furthermore, managers should harvest plants in the prototype just after the growing season to maximize B removal plants harvested then have maximum B content in their aerial tissues. Therefore, it can be suggested both plant species and vegetation distribution in the prototype are important because the management practices of plants (harvesting/exporting) may also affect the B removal capacity of the prototype.

Figure 5 shows the principal components for all plants and their location in the prototype. According to PCA statistical analyses, B and Na have the strongest relationship; B and N, Ca and Mg second; whereas B and K correlated negatively. Moreover, the statistical analyses indicated that B concentrations in the plants exhibit a strong direct relationship with Na, Ca, and N but negative correlation with K. Moreover, there was a strong statistical relationship between B and Na in the plants which are located in the inlet zone. Such relationships may be related to the increase in B mobility in the plants as a result of B toxicity (Türker et al. 2013).

Boron retention in filtration media of the prototype

The total amount of B and the extractable B (plant-available B) concentrations that are retained in the filtration media of both the prototype and the unplanted control, as well as other parameters (Ca, K, Na, N, pH, $CaCO_3$, and sediment texture) at the end of the experiment period, are shown in Table 3. The results indicated a high level of B both for total and extractable concentrations retained in the filtration media in the prototypes. Therefore, it can be concluded that using a mixture of organic-based peat and calcareous sand as a filtration material in the prototype has potential to remove or filter B from mine effluent. Some reports from literature show that B has a high affinity for organic particles, and thus B can be retained in the organic-based media through a ligand exchange mechanism related to *diol* or *cis-diol* complexes. For example, Ye et al. (2003) found 36 % removal of B using a mixture of Colma sand and organic potting medium in a microcosm experiment. Kuyucak and Zimmer (2004) reported a 47 % B removal in the natural treatment facility filled with organic peat. Allende et al. (2012) and Allende et al. (2014) suggested that organic

Table 2 Contents of B and some other elements in tissues of *T. latifolia* according to plant number and location in the prototype

		Inlet zone							Middle zone					Outlet zone						
		T_1	T_2	T_3	T_4	T_5	T_6	Average[a]	T_7	T_8	T_9	T_{10}	Average[a]	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	Average[a]
B (mg kg^{-1})	Leaves	585.2	478	259.9	478	115.5	389.8	384	148.3	319	667.9	389.3	380	702.7	724.3	721.2	712.8	299.4	512.4	611
	Stems	13.57	556.1	207.8	556.1	202.7	192.4	287	182.3	454.5	258.9	171	266	395.4	88.37	267	615.4	186	348.4	316
	Roots	281.5	342.3	474.6	342.3	385.1	114	323	284.8	277.2	251.4	242.4	263	97.23	635.5	171.2	644.7	241	66.7	309
Na (mg kg^{-1})	Leaves	3775	3368	2752	4388	2707	3186	3362	2530	2716	2496	3324	2766	4109	3310	1878	708.7	2385	2839	2538
	Stems	3582	3868	3640	3259	3477	3162	3498	3677	3970	3779	4211	3909	3626	3632	3399	185.6	4048	4204	3182
	Roots	4398	2926	4710	3449	4139	4572	4032	3198	3410	4277	3808	3673	3948	5159	3992	335	5120	3203	3626
K (mg kg^{-1})	Leaves	1263	239.1	586	783.5	338.5	475.8	614	451.5	462.3	722.1	447.1	520	1435	801.3	772.5	537	839.4	543.3	821
	Stems	2947	636.9	1516	1568	2753	1611	1838	2973	2926	1947	1543	586	3285	3074	3790	1325	3573	3142	505
	Roots	1622	1355	1669	1507	3459	1635	1874	3477	3034	3690	1584	736	3232	1383	3413	1463	3245	3140	2646
Ca (mg kg^{-1})	Leaves	7863	6129	6334	7131	5443	10,877	7296	7570	9229	11,114	5990	8475	8937	9434	1094	8548	9767	7632	7568
	Stems	5723	12,448	4855	4504	4439	5897	5571	3422	6412	50,829	7050	4232	5273	3670	216.2	27,908	13,709	3869	9107
	Roots	14,097	4060	11,263	4285	5172	7349	7704	3308	4496	6464	3156	4356	3976	5227	350	17,754	7713	10,512	6926
N (%)	Leaves	0.377	0.494	0.385	0.571	0.462	0.490	0.463	0.522	0.391	0.996	0.378	0.571	0.516	0.464	0.380	0.455	0.663	0.384	0.477
	Stems	0.671	0.446	0.653	0.792	0.544	0.471	0.596	0.507	0.505	0.377	0.456	0.461	0.118	0.662	0.477	0.371	0.596	0.568	0.465
	Roots	0.653	0.634	0.604	0.464	0.444	0.29	0.437	0.454	0.499	0.511	0.474	0.484	0.543	0.225	0.476	0.403	0.506	0.384	0.422

Superscript (a) indicates average boron and some other elements concentrations according to location in the prototype

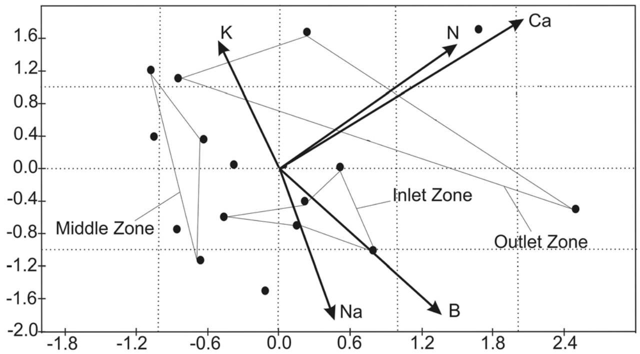

Fig. 5 Results of PCA analysis of the element concentrations within the *T. latifolia* in different locations

cocopeat substrate was the most effective filling material compared to zeolite and limestone for B removal, and organic materials content positively correlates with B removal in EWs. These results are in good agreement with our findings as sorption to organic material can be the key mechanism for removal of B both in the prototype and the unplanted control in the experiment, suggesting that using organic-based media in the prototype would improve the removal of B from mine effluent. In addition, it can be concluded that total and extractable B concentrations in the prototypes tended to increase toward the outlet, meaning that a number of the sorption sites have taken up B in the outlet zone of the prototype. Therefore, it can be emphasized that retained B concentrations in the filtration media can be controlled by other factors such as pH, porosity, particle size, and compaction. Additionally, in this present experiment, the calcareous sand was used as the filling material in the prototype in order to increase the retention of B content in the filtration media due to co-precipitation of B with calcium. As seen in Table 3, Ca concentrations and $CaCO_3$ in filtration media of the prototype were higher than the unplanted control media. This result indicated that an effective co-precipitation site formed between Ca and B in the prototype, and thus filtration media in the prototype absorbed more Ca compared to the unplanted control media during the experiment period. That is why higher Ca content in the filtration media is known to improve B removal efficiency, and this may also explain the higher B removal performance for the prototype compared to the unplanted control. Other parameters such as subsequent differences in flow regime due to plant density are considered to have a crucial role on retained B content in the filtration media. Because of the differences in the flow regime between the prototype and the unplanted control, the retention time was slightly higher in the prototype, and thus more B was filtrated from mine effluent. According to the present results, B content in the filtration media of the prototype could increase in time and, thus, the lifespan of the filtration media may only be limited by B overload. Therefore, further settling or periodic maintenance may be required if the filtration media becomes saturated. It is recommended that managers should carefully calculate the lifespan of the filtration media according to B loading dosage during the study period. Finally, other organic-based filtration materials such as dead plant substrate should be tested in terms of their performances to improve the treatment efficiency of boron in the prototype engineered wetlands.

Soil enzyme activity in the prototype

Soil enzymes such as dehydrogenase, protease, and urease in wetland matrix provide crucial information about the quantitative aspects of the quality of living dynamics related to microbial activity and microbial compositions in soil environment (Zhang et al. 2010b). Therefore, researches measure the soil enzyme activities in EW in order to clarify the relationship between treatment efficiency and enzyme activities (Kong et al. 2009; Zhang et al. 2010b). However, results on the effect of B on soil enzyme activities in EWs are still

Table 3 Chemical and physical analyses of filtration media from different locations in the prototype and unplanted control at the end of the experiment

	Prototype			Unplanted control		
	Inlet zone	Middle zone	Outlet zone	Inlet zone	Middle zone	Outlet zone
Total amount of B (mg kg^{-1})	<5	<5	120	<5	<5	97
Extractable B (mg kg^{-1})	2.97	4.57	5.77	2.06	1.91	2.15
pH (−log[H+])	8.2	8.25	8.16	7.84	7.87	7.97
EC	670	641	704	561	418	667
$CaCO_3$ (%)	51	51	55	52	51	55
N (%)	0.06	0.055	0.02	0.04	0.02	0.01
Ca (mg kg^{-1})	24,426	26,856	28,327	24,060	24,037	25,471
K (mg kg^{-1})	92	92	92	82	69	86
Na (mg kg^{-1})	135	129	133	125	120	151
Sediment Texture	Sand	Sand	Sand	Sand	Sand	Sand

lacking, and no direct research has been performed so far; therefore, this research also aims to fill these gaps in literature.

The activities of dehydrogenase, protease, and urease both in the prototype and the unplanted control based on different B mine effluent loading rates are shown in Fig. 6. It can be seen that soil enzyme activities gradually decreased with increasing B concentration in the mine effluent. Dehydrogenase enzyme activity decreased from 16.9 to 13.4 μg TPF g^{-1} h^{-1} and protease activity also decreased from 347.3 to 220.5 μg tyrosine g^{-1} 24 h^{-1} with the increase in B loading rate from 0.0084 to 0.0492 mg m^{-2} day^{-1}. A similar trend was also determined for the urease activity, and thus higher activities in the prototype were observed as 449 μg NH_4^+ g^{-1} 48 h^{-1} in the first feeding period, while the lowest activity of urease in the prototype was measured as 330.5 μg NH_4^+ g^{-1} 48 h^{-1} in the third feeding period. Therefore, the results of the present experiment, in which different doses of B had been introduced into the prototype, showed that B was an inhibitor for the activity of soil enzyme activities in the prototype. So, it can be concluded that overall microbial activity in the first feeding period is higher than the second and the third feeding period, and more oxidation site related to mineralization and transformation of organic matters occur in the prototype during the first feeding period as reported by Kong et al. (2009). It was also interesting to see that the activities of dehydrogenase in the prototype were generally higher than the unplanted control. It was probably associated with the higher supply of oxygen in the sediment in the planted prototype than the unplanted control, as macrophytes such as *T. latifolia* are known to release oxygen into the wetland matrix. In parallel, urease and protease activities in the prototype were higher than the unplanted control, indicating that the presence of plants increased urease and protease activities as it catalyzes the hydrolysis of urea to carbon dioxide and ammonium, as well as the type and amount of exudates from diverse roots in the prototype (Zhang et al. 2010b). All of these results also explained more Fe precipitation as iron plaque associated with the activities of iron-depositing bacteria in the prototype compared to the unplanted control. However, further studies are needed for a deeper understanding on the relationship between soil enzyme activities and vegetation structure in the wetland systems treating B-containing wastewater. Nevertheless, the monitoring of soil enzyme activities such as dehydrogenase, protease, and urease in wetland matrix could serve as important biomarkers for aquatic environment contaminated by B.

Quantification and evaluation of B mass balance model in the prototype

According to the results of the present experiment, we found that B removal mechanism in the prototype matrix included sorption of B on filtration media, accumulation or

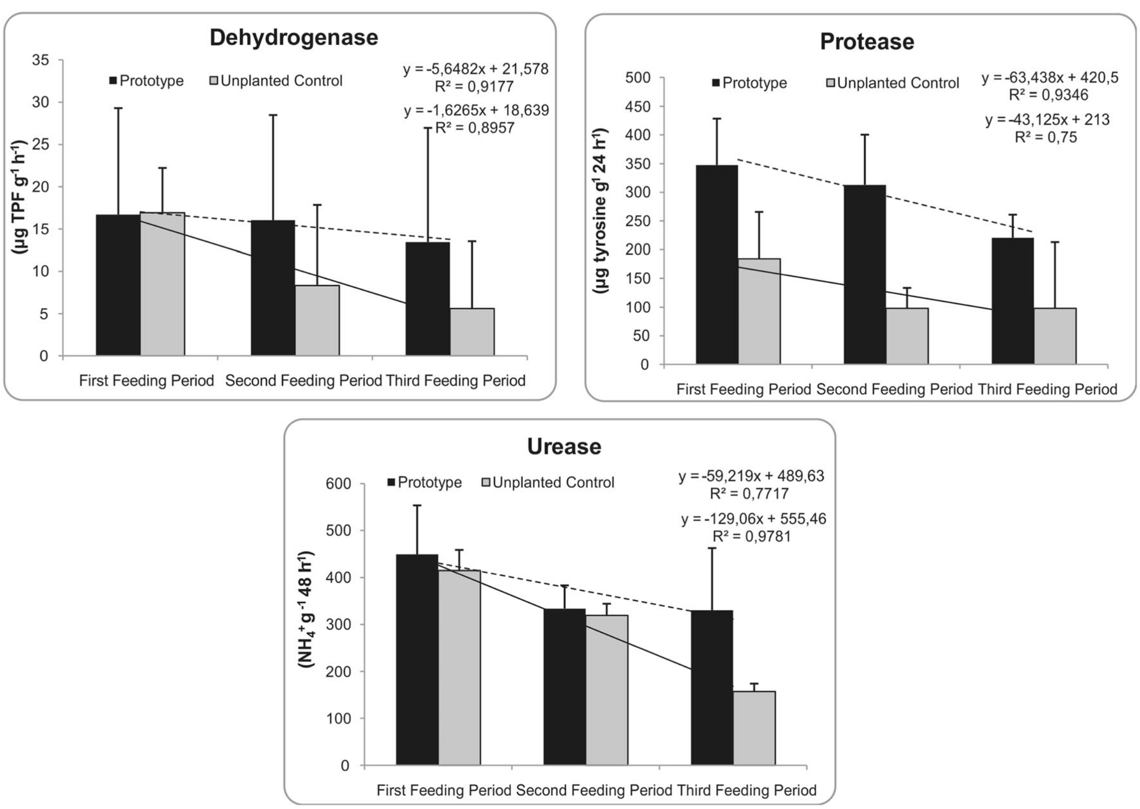

Fig. 6 Activities of dehydrogenase, protease, and urease both in prototype and unplanted control according to B mine effluent loading rates in the experiment period. *Error bars* indicate standard deviation

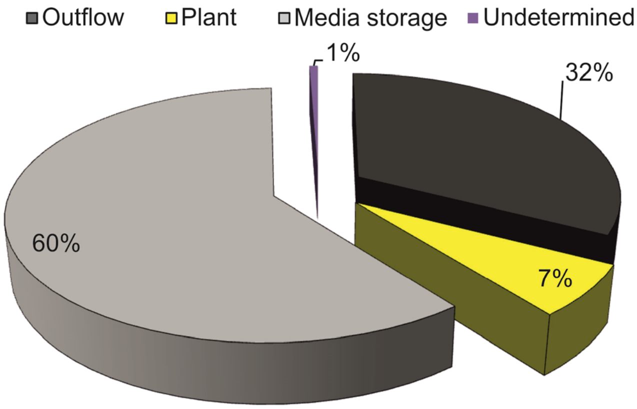

Fig. 7 Proportion of boron (B) removed by different pathways among the prototype during the experiment period

precipitation in organic-based media, and plant uptake. The first two processes have an acceptable capacity for sustainable B removal in the prototype. Proportion of B removed by different mechanisms and pathways in the prototype throughout the experiment period was calculated by mass balance model (Fig. 7). According to the model, filtration material removed 60 % of total B input, while B removed by plant uptake was 7 %. Other B removal processes such as possible microbial uptake or dead leaves loss removed around 1 % of inflow B. Therefore, it can be concluded that B removal mechanism in the prototype is based on the combined activity of filtration media and plant, as well as associated microorganism and filtration media storage. Moreover, the results also suggested that plant uptake might be among the key factors limiting B removal in EWs, but *T. latifolia* had a smaller contribution to B removal process.

Assessment of potential secondary benefits using the prototype in the mine ecological systems

Our results suggested that our prototype approach could be a *green solution* to be developed and tested by the countries with natural B deposit to reduce B mine pollution. In this respect, using a prototype wetland for B mine effluents not only provide significant help to control B mine effluent pollution but also several ancillary benefits including biogas production, green fertilizer creation from harvested plants in the prototype, and irrigation reuse of outflow.

Firstly, we propose that aboveground biomass of *T. latifolia* in the prototype is harvested periodically, and then the raw materials are converted to biogas through fermentation process. Moreover, the biogas can be used to produce electricity. Previous studies from literature have indicated that aquatic plants such as *T. latifolia* are defined as a potential substrate for biogas production because *T. latifolia* have the capability of high biomass production in natural and engineered wetlands (Cria et al. 2005; Dipu et al. 2011). With respect to aboveground biomass, results demonstrated that *T. latifolia* is an adequate raw material to be used as a substrate in biogas production process. Therefore, it can be concluded that the prototype in macrophytes can be used as an alternative energy source instead of fossil fuels in the boron mining operation. From this perspective, Table 4 shows an estimated energy budget for the prototype according to harvestable plant biomass in the present experiment.

Secondly, although we did not test potential production of green fertilizer from *T. latifolia* in the prototype, it can be hypothesized that the remaining products of the plants from the biogas production process can be used as a green fertilizer in areas with B-deficient soils in all over world.

Thirdly, the results of the present study showed that B concentrations in the treated effluent ranged from 2 to 14 mg l^{-1} during the study, and this concentration scale mostly meets water quality criteria for reuse in terms of boron concentration for irrigation purpose which can be used for agriculture (Hilal et al. 2011). Treated effluents can be suitable for some B tolerant agricultural crop reuse; thus, we suggest that the reuse of treated effluents from the prototype may bring significant environmental and economic advantages in arid and semi-arid areas which suffer from serious water shortages as B mine reserve sites.

Finally, it would require some preliminary research conducted by environmental strategists and engineers together

Table 4 Harvestable plant biomass (above-ground plant biomass) from the prototype, organic matter, and ash content in *T. latifolia*, as well as prototype estimated energy budget

Species	Harvestable biomass (DW)	Organic matter	Ash	Biogas yield[a]	Methane yield[b]	Carbon dioxide yield[c]	Energy yields[d]	Electrical energy yield[e]
	(kg m^{-2})	(kg m^{-2})	(%)	(m^3 kg^{-1} m^{-2})	(m^3 kg^{-1} m^{-2})	(m^3 kg^{-1} m^{-2})	(MJ kg^{-1} m^{-2})	(kWh kg^{-1} m^{-2})
T. latifolia	1.431	1.27	9.5	0.432	0.3934	0.0386	14.16	1.512

[a] Biogas yield from biomass according to Buswell (1952) and Kaltwasser (1980)

[b] Methane yield from biomass according to Buswell (1952)

[c] Carbon dioxide yields from biomass according to Buswell (1952)

[d] Energy yield according to Kaltwasser (1980)

[e] Electrical energy yields, 1 kWh = 3.6 MJ, according to Filimonau et al. (2011)

to design and test our prototype for different families of mine effluents including metalloid. By playing on the design parameter of the prototype, and its abiotic (filtration media) and biotic (plants, microorganism) components, energy fluxes within wetland matrix may be focused on a strategic objective related to a group of mine pollutants. For this purpose, further studies should be undertaken in order to facilitate and optimize the prototype design, and in particular more consideration should be given particularly to innovative approaches and possible secondary benefits. Therefore, we think that utilization of the prototype approach would mean applying and developing an innovative model for a deeper understanding of environment-friendly options and ecological sustainability.

Conclusions

Boron mine effluent pollution in the environment is a problem, and innovative and holistic approaches are necessary to control and manage this toxicant within mine ecological systems. The results of the present experiment suggested that the prototype EW could be a reasonable bio-filter option to control B pollution directly from mining effluent outlets in B mine reserve areas all over the world. According to the results, B concentrations in mine effluent significantly decreased with the prototype at the range between 17.5 and 5.7 mg l^{-1} after passing through the prototype. Although *T. latifolia* in the prototype showed good resistance to alterations in B mine effluent loading rates, the activities of soil enzymes such as dehydrogenase, protease, and urease gradually decreased with increasing B dosages. Therefore, we found that B was an inhibitor for dehydrogenase, protease, and urease soil enzymes in the prototype during B removal process. Moreover, based on B mass balance model, the main B removal pathway in the prototype was found as media storage (60 %), while plant has minor contribution (7 %) to the overall removal of B. Although this ecological prototype does not always meet the level of B pollution control with conventional treatment technologies, we suggested that the prototype approach can often be more economical, easier to operate, and cost effective than the energy intensive conventional treatment plants due to its potential secondary benefits.

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References

Allende KL, Fletcher TD, Sun G (2012) The effect of substrate media on the removal of arsenic, boron and iron from an acidic wastewater in planted column reactors. Chem Eng J 179:119–130

Allende KL, McCarthy DT, Fletcher TD (2014) The influence of media type on removal of arsenic, iron and boron from acidic wastewater in horizontal flow wetland microcosms planted with *Phragmites australis*. Chem Eng J 246:217–228

Böcük H, Yakar A, Türker OC (2013) Assessment of *Lemna gibba* L. (duckweed) as a potential ecological indicator for contaminated aquatic ecosystem by boron mine effluent. Ecol Indic 29:538–548

Buswell E G., 1952, “Laboratory studies of sludge digestion [Article]/ Illinois Division of State Water Survey”, Vol. Bulletin no. 30.

Chen Y, Wen Y, Zhou J, Tang Z, Li L, Zhou Q, Vymazal J (2014) Effects of cattail biomass on sulfate removal and carbon sources competition in subsurface-flow constructed wetlands treating secondary effluent. Water Res 59:1–10

Cria MP, Solano ML, Soriona P (2005) Role of macrophyte *Typha latifolia* in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel. Biosyst Eng 92(4):535–544

Debing J, Lianbi Z, Xiaosong Y, Jianming H, Mengbin Z, Yuzhong W (2009) COD, TN and TP removal of *Typha* wetland vegetation of different structures. Pol J Environ Stud 18:183–190

Dipu S, Anju AK, Thanga VSG (2011) Potential application of Macrophytes used in phytoremediation. World Appl Sci J 13:482–486

Filimonau V, Dickinson J, Robbins D, Huijbregts MAJ (2011) Reviewing the carbon footprint analysis of hotels: life cycle energy analysis (LCEA) as a holistic method for carbon impact appraisal of tourist accommodation. J Clean Prod 19:1917–1930

Gross A, Shmueli O, Ronen Z, Raveh E (2007) Recycled vertical flow constructed wetland (RVFCW)—a novel method of recycling greywater for irrigation in small communities and households. Chemosphere 66:916–923

Hilal N, Kim GJ, Somerfield C (2011) Boron removal from saline water: a comprehensive review. Desalination 273:23–35

Kaltwasser BJ (1980) Biogas-regenerative Energieerzeugung durch anaerobe fermentation organischer Abfaelle in Biogasanlagen. Bauverlag, Berlin

Kong L, Wang YB, Zhao NL, Chen ZH (2009) Enzyme and root activities in surface-flow constructed wetlands. Chemosphere. 76:601–608

Kuyucak, N., Zimmer, M. (2004) Natural systems successfully treating landfill leachate. The ISWA Roma 2004 Conference, 17–21 October, Rome, Italy

Leto C, Tuttolomondo T, Bella SL, Leone R (2013) Effects of plant species in a horizontal subsurface flow constructed wetland-phytoremediation of treated urban wastewater wity *Cyperus alternifolius* L. and *Typha latifolia* L. In the west of Sicily (Italy). Ecol Eng 61:282–291

Oertli JJ, Grgurevic E (1975) Effect of pH on the absorbtion of boron by excized barley roots. Agron J 67:278–280

Okay O, Güçlü H, Soner E, Balkaş T (1985) Boron pollution in the Simav River, Turkey and various methods of boron removal. Water Res 19(7):857–862

Özdemir M, Kıpçak İ (2010) Recovery of boron from borax sludge of boron industry. Min Eng 23:685–690

Ramilá CDP, Leiva ED, Bonilla CA, Pastén PA, Pizarro GE (2015) Boron accumulation in *Puccinella frigida*, an extremely tolerant and promising species for boron phytoremediation. J Geochem Explor 150: 25–34

Stiles AR, C. L, Kayama Y, Wong J, Doner H, R. F, Terry N (2011) Evaluation of the boron tolerant grass, *Puccinellia distans*, as an initial vegetative cover for the phytorestoration of a boron-contaminated mining site in Southern California. Environ Sci Technol 45:8922–8927

Taştan BE, Duygu E, Dönmez G (2012) Boron bioremoval by a newly isolated *Chlorella* sp. and its stimulation by growth stimulators. Water Res 46:167–175

Thakur N, Kumar SA, Shinde RN, Pandey AS, Kumar SD, Reddy AVR (2013) Extractive fixed-site polymer sorbent for selective boron removal from natural water. J Hazard Mater 260:1023–1061

Türker OC, Böcük H, Yakar A (2013) The phytoremediation ability of a polyculture constructed wetland to treat boron from mine effluent. J. Hazard Mater 252-253:132–141

Türker OC, Türe C, Böcük H, Yakar A (2014a) Constructed wetlands as green tools for management of boron mine wastewater. Int J Phytorem 16(6):537–553

Türker OC, Vymazal J, Türe C (2014b) Constructed wetlands for boron (B) removal: a review. Ecol Eng 64:350–359

Vymazal J (2013) The use of hybrid constructed wetlands for wastewater treatment with special attention to nitrogen removal: a review of a recent development. Water Res 47:4795–4811

Vymazal J, Kröpfelová L (2008) Wastewater treatment in constructed wetlands with horizontal sub-surface flow. Springer, Dordrecht

Wellburn AR (1994) The spectral determination of chlorophyll a and chlorophyll b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144: 307–313

Wolska J, Bryjak M (2013) Methods for boron removal from aqueous solutions: a review. Desalination 310:18–24

Wu H, Zhang J, Li P, Zhang J, H. X, Zhang B (2011) Nutrient removal in constructed microcosm wetlands for treating polluted river water in northern China. Ecol Eng 37:560–568

Wurl J, Mendez-Rodriguez L, Acosta-Vargas B (2014) Arsenic content in groundwater from the southern part of the San Antonio–El Triunfo mining district, Baja California Sur Mexico. J Hydrol 518:447–459

Ye ZH, Lin Z-Q, Whiting SN, de Souza MP, Terry N (2003) Possible use of constructed wetland to remove selenocyanate, arsenic, and boron from electric utility wastewater. Chemosphere 52:1571–1579

Zhang CB, Wang J, Liu WL, Zhu SX, Ge HL, Chang SX, Chang J, Ge Y (2010a) Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland. Ecol Eng 36:62–68

Zhang CB, Wang J, Liu WL, Zhu SX, Liu D, Chang SX, Chang J, Ge Y, (2010b) Effects ofplant diversity on nutrient retention and enzyme activities in a full-scale constructed wetland. Bioresource Tech 101: 1686–1692