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The Removal Efficacy of Heavy Metals and Total Petroleum Hydrocarbons from Contaminated Soils by Integrated Bio-phytoremediation

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ABSTRACT

In this study, the bio-phytoremediation and phytoremediation technologies were applied to the soils contaminated with total petroleum hydrocarbons (TPH) and heavy metals to evaluate the remediation efficacy from May 2012 to December 2013. Poplar (*Populus bonatii Levl.)* and Sun Hemp (*Crotalaria juncea L.)* were selected and planted in phytoremediation practice. These plants were also utilized in the bio-phytoremediation practice, with the addition of earthworm (*Eisenia fetida*) and petroleum-degrading bacteria (*Pseudomonos sp. NKNU01*). Furthermore, physiological characteristics, such as photosynthesis rate and maximal photochemical yield, of all testing plants were also measured in order to assess their health conditions and tolerance levels in adverse environment. After 20 months of remedial practice, the results showed that bio-phytoremediation practice had a higher rate of TPH removal efficacy at 30-60 cm depth soil than that of phytoremediation. However, inconsistent results were discovered while analyzing the soil at 100 cm depth. The study also showed that the removal efficiency of heavy metals was lower than that of TPH after remediation treatment. The results from test field tissue sample analysis revealed that more Zinc than Chromium was absorbed and accumulated by the tested plants. Plant height measurements of Poplar and Sun Hemp showed that there were insignificant differences of growth between the plants in remediation plots and those in the control plot. Physiological data of Poplar also suggested it has higher tolerance level toward the contaminated soils. These results indicated that the two testing plants were healthy and suitable for this remediation study.

Keywrods: Total petroleum hydrocarbons (TPH), Bio-phytoremediation, Phytoremediation, Earthworm, Petroleum-degrading bacteria, Poplar, Sun Hemp

1. Introduction

The contaminated site with an area of 1,800 m^2, located at Pintung County in Taiwan, was used as remediation study site due to its high amount of TPH (total petroleum hydrocarbons) and heavy metals, including zinc (Zn), chromium (Cr), copper (Cu) and Nickel (Ni), pollution under Soil and Groundwater Pollution Control Standard guideline by Taiwan Enviromental Protection Admistration. There are many available soil remediation methodologies reported in the literature, such as vapor extraction systems, soil stabilization and solidification, thermal treatments, electrokinetic systems, flushing and washing of soils, and biological methods, i.e, land farming, biopiles, bioaugmentation, and phytoremediation. Phytoremediation of heavy metals typically persisting in the environment is a low-cost and environmental friendly alternative compare to chemical techniques, therefore, has attracted increased interest since last decade (Lasat, 2002; Blaylock et al., 1997; Baker et al., 1998). Furthermore, phytoremediation has significant benefits including minimal environmental disturbance as well as without adverse effect on soil matrices. Thus, after successful phytoremediation, soil can be used directly in agriculture practices. However, phytoremediation generally removes only a very small percentage of heavy metals from contaminated soil, and can only be applied in situation with low-level

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contamination. For extremely contaminated sites, other approaches must be applied (Lasat, 2002).

Before the application of the remedial technologies on contaminated soil, the disadvantages existed in physical or chemical technoloies resulting the destruction of soil property, secondary pollutant induced and high cost, must take into consideration. For decreasing pollutants as well as responsive to maintaining the soil environment, integrated remediation such as phytoremediation, was regardes as an better technology to remove heavy metal by phytoextraction from plants like Poplar (*Populus bonatii Levl.*) *and* Sun Hemp (*Crotalaria juncea L.*) (Etim, 2012; Lorestani, 2011; Pulford and Watson, 2003; Baker et al., 2000; Raskin and Ensley, 2000; Carman et al., 1998; Critcchley, 1998; Watanabe, 1997; Cunningham and Ow, 1996). In the literatures, green remediation technology shows having an ability to remove pollutants that the related exercise on-site field to remove TPH and heavy metal was absent in Taiwan. As a result, in this study the research team had tried to testify the elimination ability of coexisted pollutants in contaminated soil by phytoremediation with Poplar and Sun Hemp and bio-phytoremediation with extra earthworm (*E. fetida*) and petroleum-degrading bacteria (*Pseudomonos sp. NKNU0*) added. The contours of pollutant concentration, including TPH and the dominant heavy metals of Zn and Cr at varied soil depths were examined in long-term opeartion from May, 2012 to Dec., 2013. Meanwhile, not only the amount of heavy metal removal by Poplar but also the growth of Poplar *and* Sun Hemp were well discussed using photosynthesis rate (PR) and maximal photochemical yield (MPY).

2. Materials and Methods

2.1. On-site description

The contaminated site is located at Pintung County with an area of 1,800 m^2. In this study field, two remedial technoligies were applied, bio-phytoremediation (BP) and phytoremediation (PR), to remove the pollution. The detailed procedure was described as the following paragraphs. With regard to area tested, two plots were selected for using BP process, classified as BP1 and BP2. However, three plots were treated by PR process, assigned as PR1, PR2, and PR3. The control plot, regarded as the uncontaminated area, was represented as CK region. All plots conducted in this study were positioned by Global Positioning System (GPS) shown in Fig. 1. Two soil samples in each plot were sampled for the measurements of pollutant concentrations. All data collected was calcualted by duplicate trials. During the remediation, the following tasks, including maintaining water content, weeding, fertilizing and preventing insect were regularly performed. For the control of watering, six sprinklers were used to evenly spray in all testing plots. Also, field operator could operate sprinklers dependent on the ambient temperature and condition of plant growth. Weeding was done by labor. The composition of fertilizer was consisted of 4.6 % of TN, 4.8% of TP, 3.2% of TK and 50% of OM. Agricultural insecticide was applied to control the insect.

2.2. Remedial technologies

In this research, the high amounts of TPH and heavy metals coexisted in contaminated area, the bioremediation with

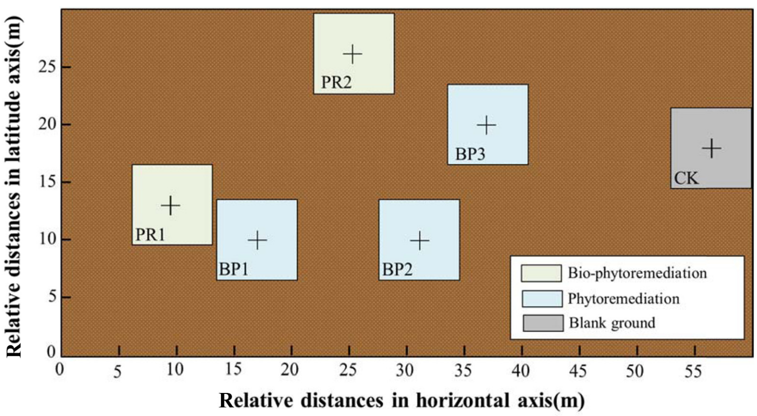

Fig. 1. Sampling locations with different remedial technologies in contaminated area.

earthworm and petroleum-degrading bacteria was used as biological processes to remove TPH (Parrish et al., 2006). Each bioremedial plot had applied with 5.4 kilograms of earthworm and 20 L of petroleum-degrading bacteria. The region for use of earthworm to treat pollutants was surrounded and laid an impermeable plastic sheet with botton layer of 60 cm and depth of 40 cm. Liquid form of petroleum-degrading bacteria was sprayed on contaminated soil by hand sprayer. On the study of phytoremediation, several researchers proved that Poplar and Sun Hemp had an excellent tolerance with high TPH and heavy metal coexisted in contaminated soil (Euliss et al., 2008; Collins, 2007; Huang et al., 2005). In Taiwan, due to the lack of imfomation to remove coexisted pollutants by plant, the *Populus bonatii Levl.* and *Crotalaria juncea L.* were selected to establish an operational procedure in on-site phytoremediation study. The distance of Poplar planted was 2.5 m apart. Sun Hemp was planted in between Poplars planted.

2.3. Parameters analysis

2.3.1. Total petroleum hydrocarbons (TPH)

Contaminated soils either prepared in the laboratory or collected in situ were used in this study. Soil samples collected from surrounding region were sieved by a 2-mm mesh sieve to remove large particles, and then the sample was homogenized in a stainless steel container by a mixer. Soil sample from 60 cm depth in PR1 plot could not be collected due to the previous existed underground cement layer. The target pollutants of spiked soils were added with a 1 : 1 (v/v) n-hexane/acetone solution and placed in a fume hood to eliminate solvents. The TPH analysis was according to standard method of NIEA S70361B by Environmental Protection Adminstration (EPA) of Taiwan. The TPH concentration was mesured by GC-FID (HP-6890 series, USA) with the calibration curve produced from spiked CK plot soil sample with various concentration of TPH standards.

2.3.2. Heavy metal

Before the analysis of heavy metals in soil samples, the same procedure of pretreatment with TPH was applied and digested by concentrated HNO_3 and HCl (1 : 3, v/v) (aqua regia). As the plant biomass heavey metal measurement, including plant leaves and root, the mixed acid solution from HNO_3 and $HClO_4$ with a ratio of 5 was used to digesting the whole samples pretreated by roasting and crumbling. The digested solutions were filtered by using Whatman No. 42 filter paper and diluted to adequate volume with deionized water. The heavey metal analysis was completed with a flame atomic absorption spectrophotometr (FAAS) (Hitachi Z-8100, Japan) in accordance with NIEA S361.63 method of EPA of Taiwan.

2.3.3. Physiological investigation

Net photosynthetic rate and maximal photochemical yield (F_v/F_m) of chlorophyll fluorescence in dark-adapted samples of Poplar were measured. During the study period, duplicate measurements in each sample were collected. As well known, photosynthesis is an important physiological activity of plants. Its efficiency reveals the physiological condition of a plant. Four samples from each plant of the 6 treatment plots were randomly selected. Two leaves from each sample were collected and measured. The measurements were taken between 7 : 30 to 10 : 30 am, using a portable photosynthesis system (LI-6400, LI-COR, USA). Microenvironment in the leaf chamber was set at 1,500 μmol photon m^{-2} s^{-1} of light, 400 μL L^{-1} of CO_2 concentration, 60-80% of relative humidity, and 28°C of leaf temperature. Net photosynthetic rates obtained under these fixed conditions can be used to compare the physiological status of Poplar planted in different treatment plots.

Since chloroplast is the part for photosynthesis, measuring maximal photochemical yield (F_v/F_m) of chlorophyll fluorescence in a dark-adapted leaf can indicate the health condition of a plant. A portable chlorophyll fluorescence analyzer (Mini-PAM, Walz, Germany) were used for this measurement. The process of dark adaptation was preceded by covering the leaf with a dark leaf clip (DLC-8) for 30 minutes. Then we measured the minimum (F_o) and the maximum (F_m) fluorescence values of this dark-adapted leaf, and calculated its F_v/F_m value (where $F_v = F_m - F_o$). In plant physiology, F_v/F_m represents the maximal photochemical potential of photo-systems II. It can be used as an index for indicating whether the plant is under stress, therefore, it was used to evaluate a plant's health condition.

Table 1. Water content and organic matter in different depths of studied soils

	Water contents (%) in different depths (cm)			Organic matter (%) in different depths (cm)		
Regions	30	60	100	30	60	100
BP1	24 ± 1.4	26 ± 2.8	36 ± 2.8	8 ± 1.0	11 ± 3.8	24 ± 0.1
BP2	20 ± 2.1	27 ± 2.8	27 ± 2.8	5 ± 1.2	6 ± 3.7	6 ± 3.7
BP3	27 ± 2.1	27 ± 0.7	32 ± 0	10 ± 1.5	9 ± 0.6	4 ± 0.3
PR1	35 ± 0.7	38 ± 0.7	ND	13 ± 3.0	14 ± 5.4	ND
PR2	23 ± 1.4	24 ± 2.1	26 ± 0.7	10 ± 5.7	12 ± 12.2	4 ± 0.1
CK	20 ± 0	23 ± 0.7	25 ± 0.7	3 ± 1.4	3 ± 0.2	2 ± 0.2

3. Results and Discussion

3.1. Contents of TPH in various soil depths

Table 1 shows the contents of water and organic matter in contaminated soil in earlier remediation. Based on Table 1, it showed that most of treated regions using different remedial strategies had higher water content than control region at different depths. The water contents in tested region were ranged from 20 ± 0 % to 38 ± 0.7% by the controlled irrigation schedule. Regarding the amount of organic matter, the values in CK plot value at different depths were less than those in remedial treated regions. High organic matter in remedial region than agricultural soils may be contributed to the use of organic fertilizer and TPH pollutant. Hence, low organic matter in CK region may be related with less TPH concentration.

Fig. 2 shows that the variations of TPH contour at varied depths in contaminated soil using different remedial technologies during long-term study. Observed TPH contours on May, 2012 represent early stage in the on-site area with pollutant (Fig. 2 A) the highest contaminated locations were appeared in BP1 and BP3 in 60 cm of soil depth similar to those in 120 cm of soil depth. The peak location at 30 cm of soil depth had a minor difference from BP1 and PR2 to BP1 and BP3. Actually, both plots of PR2 and BP3 were adjacent zone, indicating that source TPH in early stage was located among these plots.

After ten months remediation (Feb., 2013), BP1 and BP2 plots with bio-phytoremediation at 30 cm of soil depth showed higher TPH removal rate than PR1 and PR2 with phytoremediation. Three plots treated with bio-phytoremediation, only BP3 showed a weaker TPH removal than two plots with phytoremediation. In summary, TPH removal by

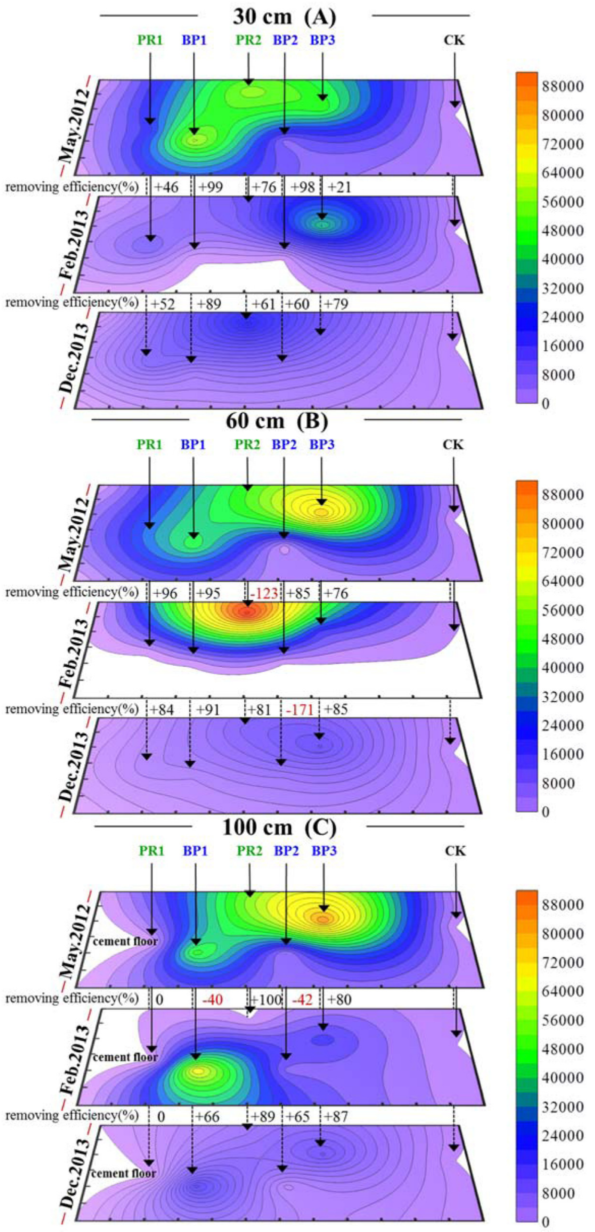

Fig. 2. Variation of TPH contents at various depths (A) 30 cm (B) 60 cm (C) 100 cm.

bio-phytoremediation was higher than that by phytoremediation. The exceptional plot of BP3 may be contributed from the heterogeneous sample, the slope of topography, and the adaptability of earthworm, petroleum-degrading bacteria, Poplar, and Sun Hemp. On Dec., 2013 after twenty months operation, part of plots showed the decrease of TPH removal efficiency compared with those on Feb., 2013, but the total trend for TPH removal by bio-phytoremediation was higher than that by phytoremediation. Only BP3 out of three plots treated by bio-phytoremediation on Dec., 2013 had a value close to that of PR2 by phytoremediation. This phenomenon was also found as TPH removal at BP3 by bio-phytoremediation compared with PR on Feb. 2013.

For 60 cm of soil depth, TPH removal at different remedial technologies in the middle stage on Feb. 2013 (Fig. 2B), high removals were found at four plots including PR1, BP1, BP2 and BP3 except for PR2 plot with TPH increase. Bio-phytoremediation revealed it's more consistent on TPH removal than phytoremediation, even though, on Dec., 2013, similar trend was observed. With regard to the soil depth of 100 cm after twenty months operation (Fig. 2C), the whole TPH removals were less than 90%; however, the ineffective removal was happened in PR1 because samples could not be collected in underground cement layer of this depth. At soil depths of both 60 cm and 100 cm, the highest TPH concentration in early stage had same region located at the BP3 plot.

Summarized from above statements, the high TPH removal in bio-phytoremedaition treatment may be attributed with simultaneous effects not only by microbial activity (Cassidy et al., 2001; Cavalca et al., 2000) and earthworm (Parrish et al., 2006) but also by polar (Euliss et al., 2008; Collins, 2007).

3.2. Contents of heavy metals in various soil depths

TPH removal in the polluted soil by bio-phytoremediation and phytoremediation was well discussed in the above paragraph. In fact, the contaminated soil conducted in this research was not only infected by oil but also by heavy metals. According to the investigation in this research (not shown), both zinc (Zn) and chromium (Cr) elements were main heavy metals. Hence, the following discussion was focused on both metals removals in long-term remediation,

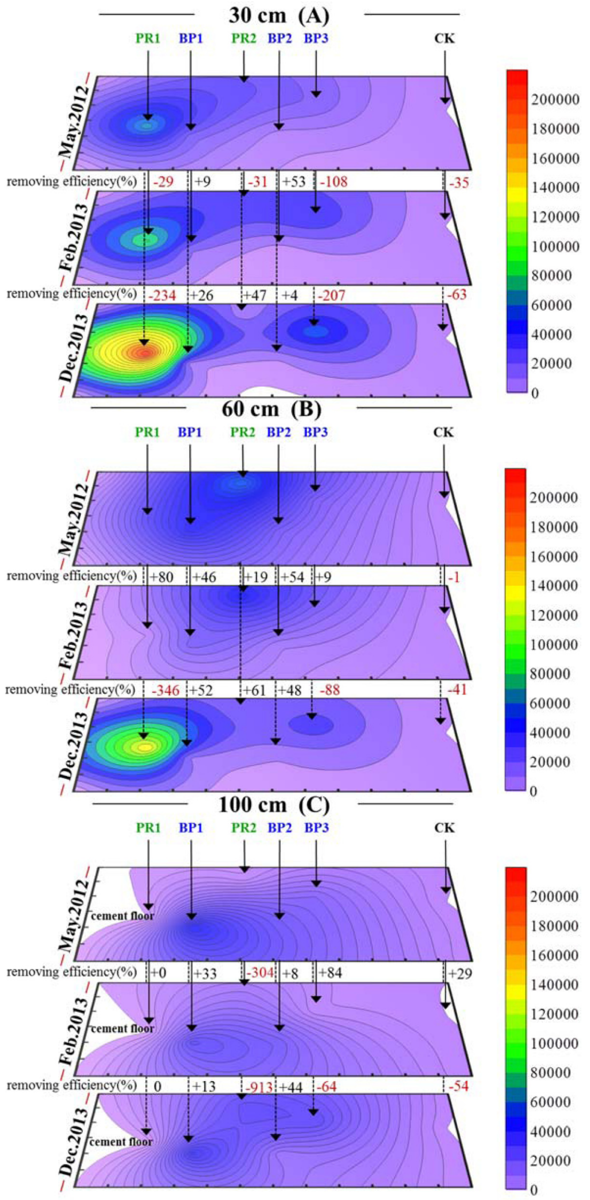

Fig. 3. Variations of Zn contents at various depths (A) 30 cm (B) 60 cm (C) 100 cm.

as shown in Fig. 3 and Fig. 4.

Zn contours at varied depths in contaminated soil by on-site remediation during long-term operation are shown in Fig. 3. In early remedial stage on May, 2012, only one peak location was observed at PR1, PR2, and BP1 for 30 cm, 60 cm and 100 cm of soil depths. The concentration of Zn was decreased with soil depth. The plot of main peak was so close in this contaminated area, indicating that high metal concentration was existed in these areas owing to inhomogeneous distribution of soil sampling. Zn removal in 30 cm

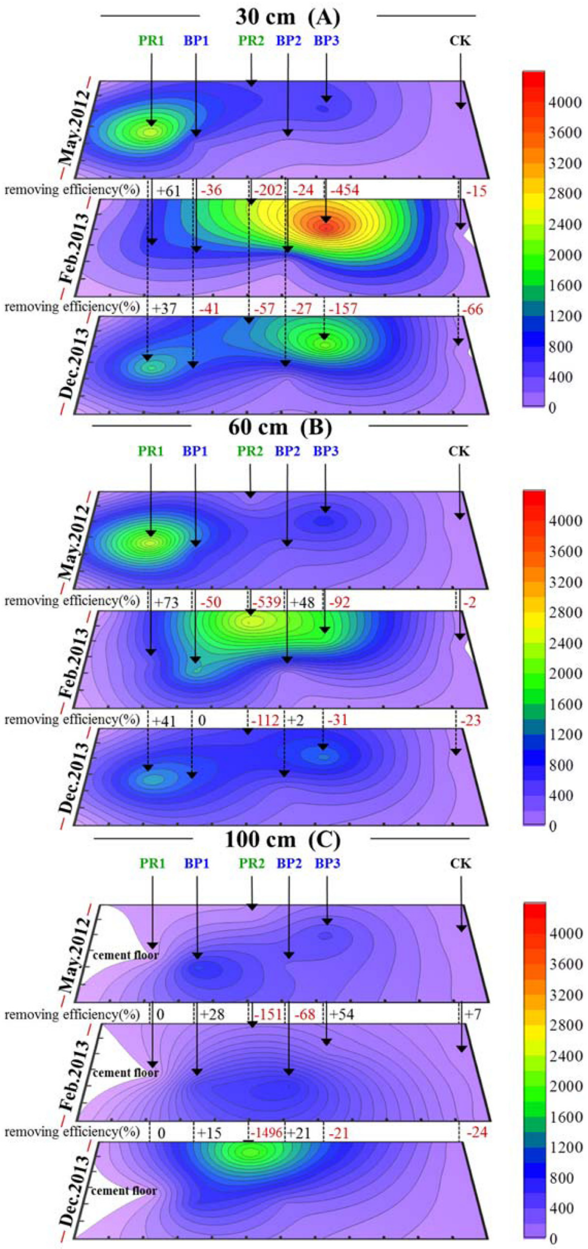

Fig. 4. Variations of Cr contents at various depths (A) 30 cm (B) 60 cm (C) 100 cm.

of soil depth on Feb. 2013, two out of three plots by bio-phytoremediation showed the value from 9% to 53%; however, BP3 and other two plots with phytoremediation showed the inability to eliminate the heavy metal. Long-term operation till Oct. 2013, the same plots by bio-phytoremediation on Zn removal was changed from 4% to 26% and the PR2 plot with phytoremediation had 46% of Zn removal. PR1 and BP3 plots have continue to show an inefficient to eliminate heavy metal, especially for PR1 with the increasing concentration of Zn element. This phenomenon was also happened in the soil depth of 60 cm. At soil depth of 100 cm, plots with bio-phytoremediation, including BP1 and BP2, eliminated 13-44% of Zn element in contrast with the increase of 64% at BP3 plot. The plot of PR2 by phytoremediation showed the insignificant variation owing to the existence of cement floor.

As for Cr contours at varied depths in contaminated soil by on-site remediation during long-term operation, its variation was shown in Fig. 4. In early remedial stage on May, 2012, peak locations for different soil depths were located PR1 plot for 30 cm, PR1 and BP3 plots for 60 cm, and BP1 and BP3 plots for 100 cm, respectively. The Cr concentration of peak location was at 30 cm and 60 cm of soil depths which are higher than that in 100 cm of soil depth. The penetration of Cr element in contaminated soil reaches 60 cm at early stage in this study. After ten and twenty months' operation at 30 cm of soil depth, Cr removal changing from 61% to 37% was found in PR1 plot by phytoremediation. Only one-fifths tested plots showed the ability to decrease the heavy metal Cr. For Cr removal at 60 cm of soil depth after twenty months' operation, PR1 plot with phytoremediation reached 41% and BP2 plot by bio-phytoremediation had lowest efficiency of 2%. Only two-fifths of remedial plot was able to remove Cr element. For Cr removal at 100 cm of soil depth after twenty months' operation, BP1 and BP2 plots by bio-phytoremediation were 15% and 21%, respectively.

Generally, the mobility for Zn is higher than Cr in subsurface could affect evapotranspiration of plant to absorb and migrate heavy metal from soil water to plant leaves via plant root system (ITRC, 2009). Actually, Kabata-Pendias (2010) proved that the removal ability of heavy metal by most of plants is from large to small was in the order Cd, Zn, Hg, Cu, Pb, As, Ni and Cr. Apparently, experiments conducted in this research show its rationality.

3.3. Characteristics of plant growth and physiology during remediation

The heights of Poplar and Sun Hemp grwon in different remedial plots were measured on Apr. and Aug. in 2013, respectively, as shown in Fig. 5. Figure 5A revealed that the heights of Poplar grown in contaminated plot compared

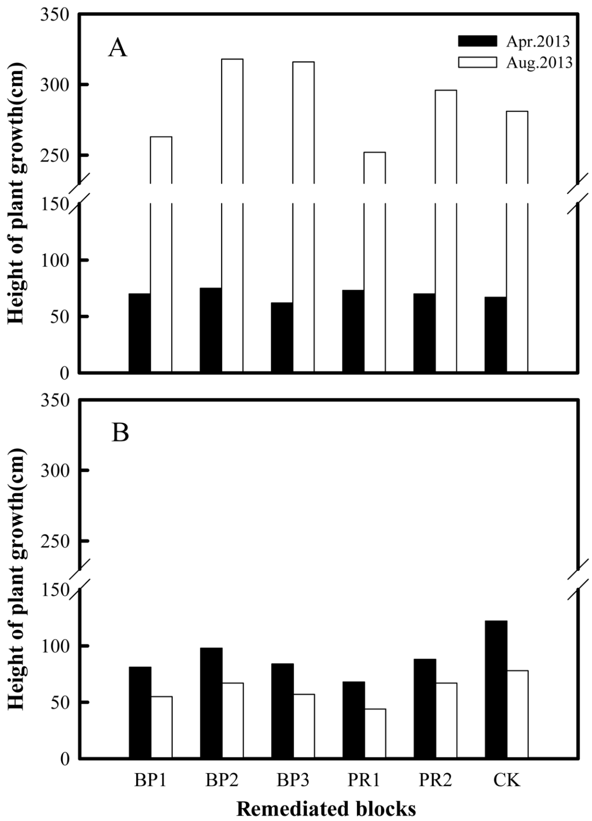

Fig. 5. Growth heights of (A) Poplar (B) Sun Hemp on Apr. and Aug., 2013 at different plots with various remediations.

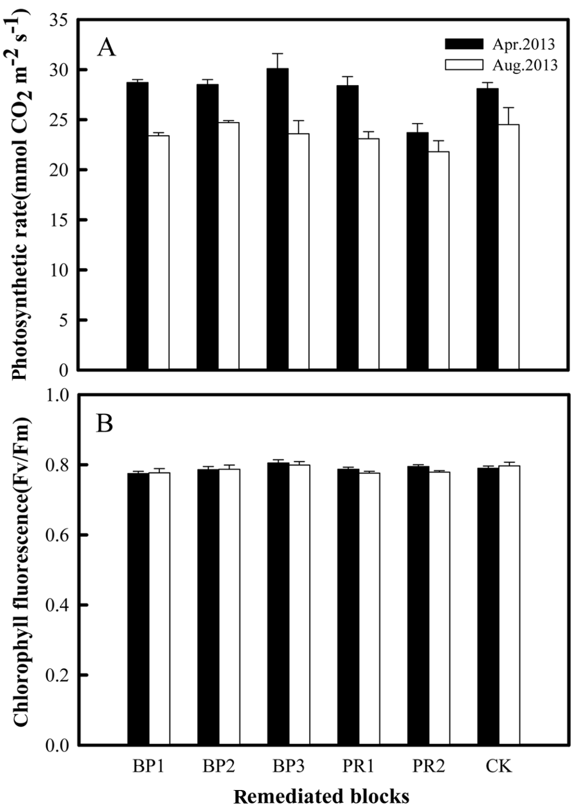

Fig. 6. Variations of (A) Photosynthesis rate (B) chlorophyll fluorescence of Poplar grown in remedial regions on Apr. and Aug. in 2013.

with control plot were similar at duplicate sampling, indicating that Poplar had high tolerance with high amount of TPH and heavy metal. Observed from Fig. 5B, the growh of Sun Hemp showed an different trend relative to that of Poplar. Apparently, high amounts of TPH and heavy metal could affect the growh of Sun Hemp.

According to above discussion, Poplar was proved to have high tolerance with high coexisted pollutants. More evidence was shown based on physiological investigation including net photosynthetic rate and maximal photochemical yield, as shown in Fig. 6. Observed from Fig. 6A, most of net photosynthetic rates of Poplar on different remedial plots compared with control plot at same date were ranged from 28.1 μmole CO_2 m^{-2} S^{-1} (CK) to 30.1 μmole CO_2 m^{-2} S^{-1} (BP3) excluding the 23.7 μmole CO_2 m^{-2} S^{-1} (PR2) on Apr. 2013. In the literature, Critcchley (1998) showed that less than 0.75 of Fv/Fm represented the phenomenon of inhibition on plant resulted from adverse circumstance. At the same remedial plot, the net photosynthetic rate measured on Aprl. 2013 was higher than that on Aug. 2013, it may be attributed with the variation of season. As for the duplicate measurments of Fv/Fm on Apr., 2013 and Aug., 2013, those values revealed insignificant difference with larger than 0.75, indicating that Poplar grown in high polluted region had better physiological performance.

3.4. Beneficial evaluation of remediation

According to previous discussion, TPH removal was higher than heavy metal removal after remediation. Fig. 7 shows the amounts of both heavy metals removals by Poplar and Sun Hemp. The leavies of Poplar had higher removal efficacy on Zn element than Cr element. Poplar grown in control plot had the lowest amount of metal removal than other remedial plots (Fig. 7 A-1 and Fig. 7 B-1). Similar trend for the amounts of Zn and Cr removal was happened in the root and shoot fractions for Poplar, as shown in Fig. 7 A-2, Fig 7 A-3, Fig. 7 B-2 and Fig. 7 B-3. In consideration of plant biomass with dried weight, the amount of heavy

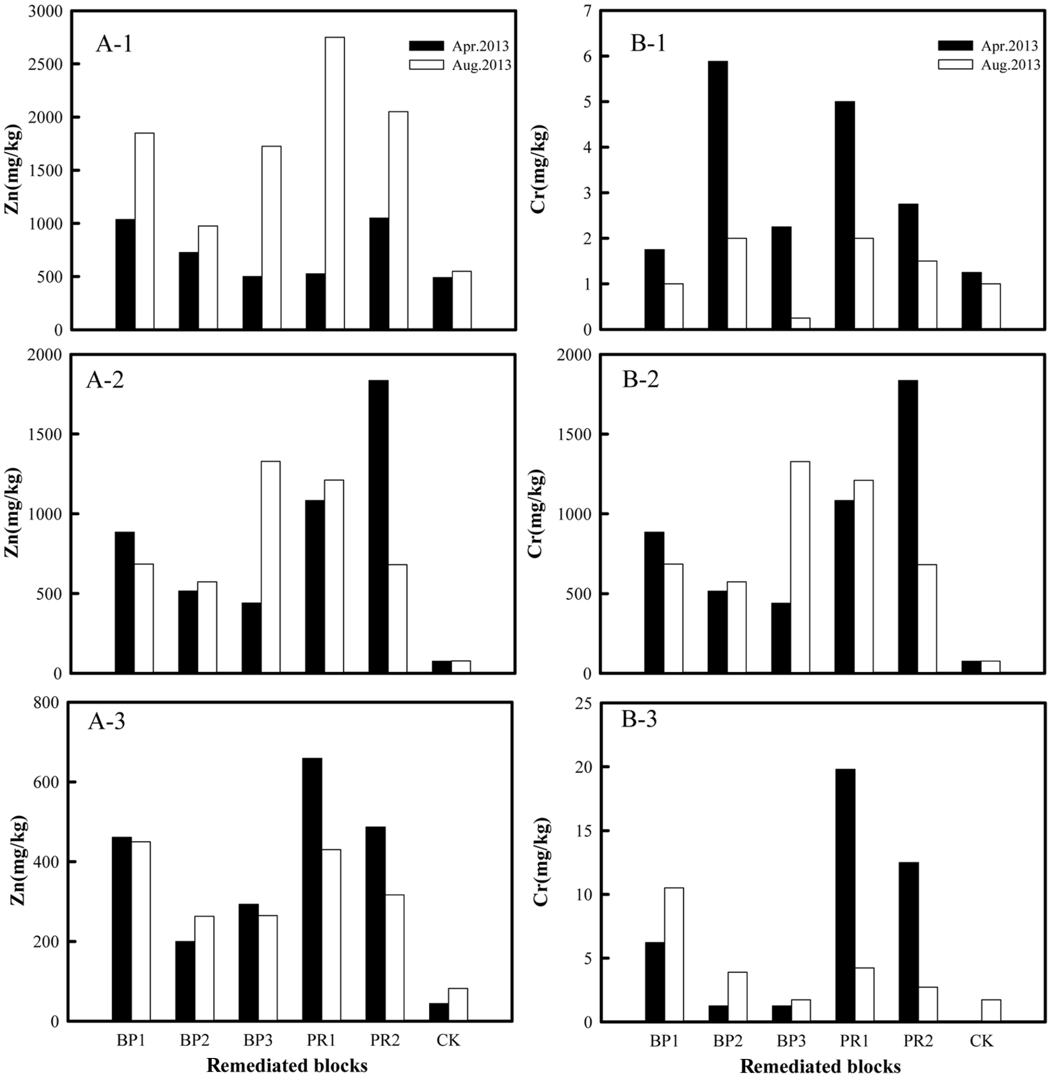

Fig. 7. Amounts of (A) Zn (B) Cr removal by (1) the leaf of Poplar (2) the root fraction of Sun Hemp (3) the shoot fraction of Sun Hemp on Apr. and Aug., 2013.

metal removal by Poplar, calculated from Fig. 7, was respectively 33.2 g for Zn and 0.048 g for Cr during the remediation, as shown in Fig. 8A. Figure 8B shows the amount of Zn and Cr removal by Sun Hemp was respectviley 50.8 g and 0.331 g. Summing up of heavy metals removal by Poplar and Sun Hemp, the amounts of elimination for Zn and Cr were 84 g and 0.38 g, respectivley, far less than the original amounts of Zn with 1,084 kg and Cr with 47.0 kg. For promoting the benefit of remediation, the frequency of sowing Sun Hemp could be considered in this field. Evidently, the growth of Poplar will gradually increase the amounts of the pollutant removals. Of course, the derivative issue, high metal concentration of leaves of polar must be collected and treated according to the regulation of local government.

Conclusions

Several objectives were completed in this research and summarized as the followings. In conclusion, the removal efficiency of TPH by both remedial technologies was higher than that of both investigated heavy metals Zn and Cr. Based on tested physiological results, Poplar shows a higher tolerance in adverse growth condition. The bio-phytoremediation and phytoremediation on metal removal efficacy was low in the case of high concentration heavey metal con-

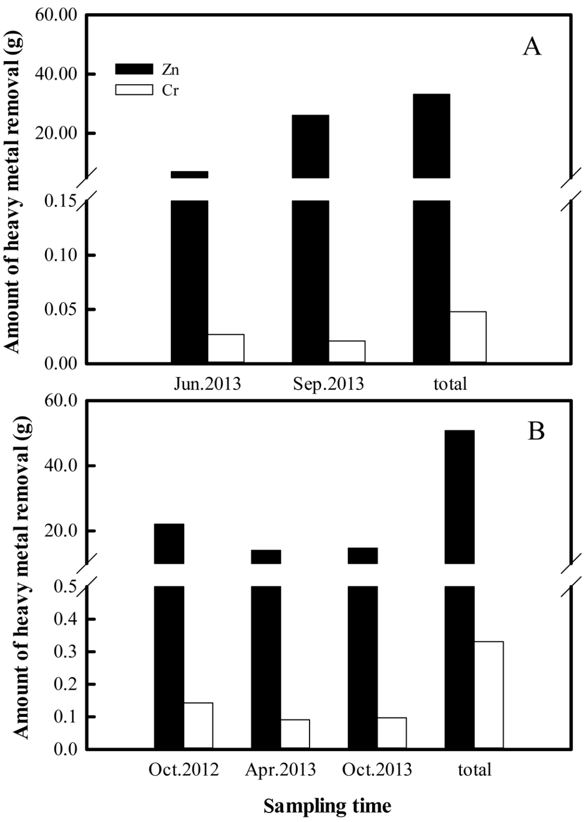

Fig. 8. Removals of heavy metals by (A) Poplar (B) Sun Hemp during remediation period.

tainminated soil. For example, the amount of Zn removal by Poplar and Sun Hemp was respectively measured in 84 g and 0.38 g after remedial treatment. In comparison with the estimated total containminated Zn amount of 1,084 kg, apparently, a long remediation period to eliminate Zn must be invested. In order to obtain the benefit from bio-phytoremediation on the elimination of heavy metals, higher frequency of Sun Hemp planted and larger biomass of Poplar continuously grown in long-term operation will significantly shorten the time of remeidation.

Acknowledgments

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