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Determination of the DNA changes in the artichoke seedlings (*Cynara scolymus* L.) subjected to lead and copper stresses

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ABSTRACT

This study aims at determining the effects of lead (Pb) and copper (Cu) on the hyperaccumulator artichoke. The effect of Pb and Cu toxicity with different levels of concentrations (20, 40, 80, 160, 240, 320, 640 and 1280 ppm) caused a decrease in the root length and total soluble protein of the artichoke. As a result of treatment with the Pb and Cu solutions, the changes occurred in RAPD profiles of seedlings and revealed variations like increment and/or loss of bands compared to the control plants. These changes showed a decrease in genomic template stability (GTS, changes in RAPD profile) caused by genotoxicity. RAPD data and GTS values seemed consistent with the results of the root length measurements and total soluble protein analysis. In addition, it was seen that the genomic template stability was significantly affected by direct proportion of primary root length, root dry weight and root total soluble protein content in artichoke subjected to Pb and Cu stresses. As a result, it can be concluded that RAPD analysis based on the used primers in the current study can be applied in combination with physiological and biochemical parameters to measure genotoxic effects of lead and copper on artichoke plants.

Keywords: heavy metals; random amplified polymorphic DNA; plant adaptation; abiotic stress

Plants growing on heavy metal-contaminated soil can accumulate toxic concentrations of these elements and reveal a critical health risk to consumers via the food chain (Chen et al. 2014). Although copper is an essential element, its high concentrations (depending on the species of plants) have toxic effects on plants. On the other hand, although lead is not an essential element, it always has toxic effects on plants. Excess copper and the existence of lead negatively affect the growth of roots, stems and leaves of plants (Zengin and Munzuroğlu 2004). Moreover, lead and copper ions may bind to thylakoid membrane proteins with high concentrations and this may lead to the inhibition of electron flow and emergence of reactive oxygen forms by damaging the structures of photosynthetic membrane (thylakoid membrane) (Pourrut et al. 2011).

The effects of lead and copper on DNA occur directly or indirectly. These can be the destabilization of the double helical structure of DNA (Anastassopoulou 2003), mismatches of the bases on nucleic acids (Eichhorn et al. 1985), single-nucleobase lesions on DNA, single DNA strand breaks, double DNA strand breaks and ion connections within strands and also degree of methylation of DNAs (Cadet et al. 2012, Karan et al. 2012). In brief, heavy metals, even if indirectly, cause oxidative DNA damages and chromosomal abnormalities (Ritambhara and Girjesh 2010).

DNA fingerprinting techniques like random amplified polymorphic DNA (RAPD) were used to determine the impacts of environmental factors on some plant samples such as barley, beans, lichen and maize (Liu et al. 2005, Cenkci et al. 2009, Cansaran-Duman et al. 2011, 2012, Batır et al. 2015).

As it can be inferred from the studies above, studies about genotoxicity that address the effects

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of environmental stress on plants have increased in recent years. However DNA changes under lead and copper stresses in artichoke have not been explored yet. In the light of this knowledge, this study aims at determining the effects of lead and copper on the genomes of hyper accumulator artichoke, which is a significant nutritional source and a precious plant as it has antioxidant characteristics.

MATERIAL AND METHODS

Plant material, growth and total soluble protein content. 85 cells of the seedling trays were filled with sterilized peat to the surface layer of tray and uniform sterilized artichoke seeds were planted in each cell of the seedling tray. The seedling trays were divided into 17 groups. Each group, control, and eight different concentrations of each lead (Pb) and copper (Cu) solution, had 5 cells. Each group of the trays were irrigated with 15-mL distilled control water (C), 15 mL for each 20, 40, 80, 160, 240, 320, 640, 1280 ppm concentrations of $Pb(CH_3COO)_2 \cdot 3\ H_2O$ lead solutions, and 15 mL for each 20, 40, 80, 160, 240, 320, 640, 1280 ppm concentrations of $CuCl_2 \cdot 2\ H_20$ copper solutions, respectively. Each seedling treatment has three replicate. Following the 14 days of treatment, the root elongation, dry weight and total soluble protein of the artichoke seedlings were measured. Total soluble protein of the artichoke seedlings were measured according to the Bradford method (Bradford 1976).

DNA extraction and RAPD procedures. At the end of the treatment, 100 mg of the primary seedling roots was washed with sterilized water and then ground with liquid nitrogen in 1.5 mL tubes. In pursuit of grounding the roots, total genomic DNA isolation of seedling roots, RAPD-PCR and electrophoresis procedures were performed according to the protocol of Batır et al. (2015). In this study, 23 decamer RAPD primers (OPA-03; OPB-08; OPC-01; OPC-02; OPC-03; OPC-04; OPC-05; OPC-06; OPC 07; OPC-08; OPC-09; OPC-10; OPC-11; OPC-12; OPC-13; OPC-14; OPC-15; OPC-16; OPC-17; OPC-18; OPC-19; OPC-20; OPO-04) were used for PCR reaction. All PCR reactions were carried out in duplicate.

Data analysis and the estimation of genomic template stability. Polymorphisms in RAPD profiles included disappearance and appearance of bands in comparison with the control and the average was calculated for each test group exposed to different concentrations of Pb and Cu. To compare the sensitivity of each parameter, length, dry weight and total soluble protein content of roots were calculated as a percentage of the control (set to 100%). Genomic template stability (GTS) was calculated as:

$$GTS = (1 - a/n) \times 100$$

Where: a – RAPD polymorphic profiles in each sample; n – number of total bands in the control. Changes in the RAPD patterns were expressed as a decrease in GTS, a qualitative measure showing the obvious change in the number of RAPD profiles generated by the artichoke samples exposed to Pb and Cu, in relation to profiles obtained from the artichoke and control seedlings.

Statistical analysis. The SPSS (statistical package software v. 15.0 for Windows) was used to analyse the changes in root length, dry weight, and total soluble protein content. Data were tested by performing the paired sample *t*-test.

RESULTS AND DISCUSSION

The effect of Cu and Pb on physiological and biochemical parameters. The accumulation of metals in plants had more negative effects on roots and the germination of seeds than it had on leaves and stem; it was reported in the seedlings of bean (*Phaseolus vulgaris* L.) that was exposed to the increasing concentrations of lead (Pb) and copper (Cu) (Zengin and Munzuroğlu 2004). Similarly, as expected from the hyperaccumulator artichoke plant, the findings of this study on the length of artichoke seedlings' roots are parallel to the previous findings in the related literature. In other words, as the concentrations of lead and copper increased, the root length of the artichoke seedlings decreased. Decreases between 0.9–47% and 1.2–80% in the root length of the artichoke seedlings that were exposed to different concentrations of lead and copper, respectively, were determined compared to the control group. Nevertheless, statistically significant differences for both lead and copper were observed above 20 ppm ($P < 0.05$) (Tables 1 and 2). A gradual decrease was determined in artichoke seedlings after 20 ppm depending on the increasing concentrations of

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Table 1. Artichoke seedlings are exposed to various lead (Pb) concentrations (mg/kg). Changes in the root lenght (cm/seedling), dry weight (g/seedling) and total protein content (mg/mL)

	C	Pb 20	Pb 40	Pb 80	Pb 160	Pb 240	Pb 360	Pb 640	Pb 1280
Root length	10.7	10.6	10.5*	10.1**	9.0**	7.7**	7.3***	7.0***	5.7***
Dry weight	0.0036	0.0036	0.0035*	0.0033*	0.0030**	0.0026***	0.0024***	0.0022***	0.0019***
Total protein content	0.033	0.033	0.032**	0.031***	0.030***	0.030***	0.029***	0.028***	0.025***

n = 14 for each group. *P < 0.05; **P < 0.01; ***P < 0.001

lead and copper (P < 0.05). With 160 ppm concentration, lead and copper had similar negative effects on the root length of the artichoke seedlings (P < 0.05). However, above 160 ppm, copper had more negative effects on the root length of artichoke seedlings compared to lead. 1280 ppm concentration of lead and 240 ppm concentration of copper had similar negative effects on the artichoke seedlings (P < 0.05). Besides, although the artichoke seeds germinated with all concentrations of lead, no germination was observed with copper's concentrations of 640 ppm and 1280 ppm.

The oxidative damage caused by divalent metal cations (Pb, Cu, Ni, Co, Zn, Mn and Cd) on mitochondria, chloroplast membranes and structure of chlorophyll acts as inhibitor on the transportation of electrons and oxidative phosphorylation; and as a result, it leads to a negative effect on the production of dry weight, in other words, causing the formation of carbon composites (Dixit et al. 2002). Compared to the control group, the exposure of lead and copper resulted in a decrease in artichoke seedlings' dry weight between 0.9–48% and 0.9–79%, respectively. The statistical discrepancy between the treated artichoke samples and the non-treated control group was observed at 20 ppm concentration for both lead and copper (P < 0.05) (Tables 1 and 2). The negative effect was observed more on the samples that were exposed to 240 (58%) and 320 ppm (79%) copper stress (P < 0.001) (Table 2). Also, there is a consistent relationship between the dry weight and length of the heavy metal treated root. R^2 value of the dry weight and root length for increasing concentrations of Pb and Cu treatment were 0.993 and 0.998, respectively (Figures 1 and 2).

Similar to the decreases in the length and dry weight of root, total soluble protein contents of all the artichoke seedlings decreased due to the increase in heavy metal concentration. Similarly, it was indicated that the increase in the concentrations of lead, cadmium, zinc and copper caused a decrease in the total protein content of sunflower and cucumber, respectively (Kastori et al. 1992, Soydam et al. 2012). Similar to the previous findings in the related literature, a negative correlation between the total soluble protein content and metal concentration was determined. A decrease between 0.9–23% in the total soluble protein content of the artichoke seedlings that were exposed to the stress of lead and a decrease between 1.2–35% in the total soluble protein content of the artichoke seedlings that were exposed to the stress of copper were determined. On the other hand, no statistically significant difference in the total soluble protein content of the artichoke seedlings was determined with the concentrations of lead and copper until 20 ppm (P < 0.01) (Tables 1 and 2).

Table 2. Artichoke seedlings are exposed to various copper (Cu) concentrations (mg/kg). Changes in the root lenght (cm/seedling), dry weight (g/seedling) and total protein content (mg/mL)

	C	Cu 20	Cu 40	Cu 80	Cu 160	Cu 240	Cu 360
Root length	10.7	10.6	10.4*	9.8**	8.6**	4.6***	2.1***
Dry weight	0.0036	0.0036	0.0035*	0.0033*	0.0028**	0.0015***	0.0008***
Total protein content	0.033	0.033	0.031**	0.028***	0.026***	0.025***	0.021***

n = 14 for each group. *P < 0.05; **P < 0.01; ***P < 0.001

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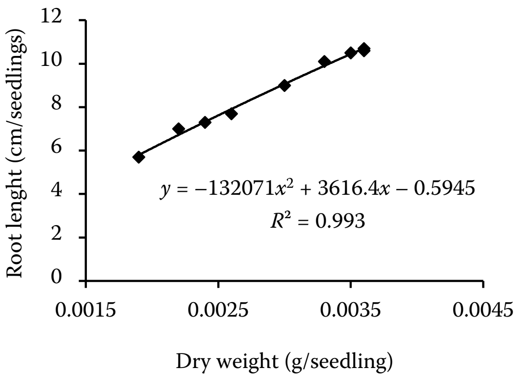


Figure 1. Influence of root dry weight to primary root length (Pb)

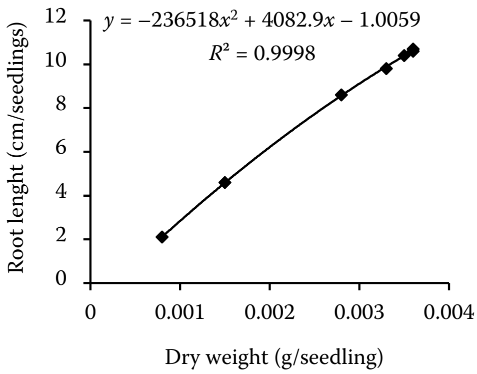
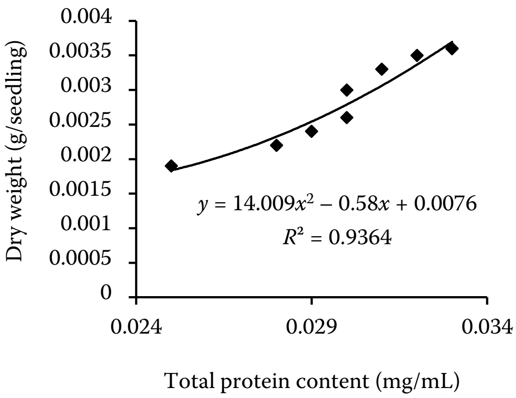


Figure 2. Influence of root dry weight to primary root length (Cu)

At the same time, positive correlations were observed between root total soluble protein content and root dry weight (Figure 3) as well as between root total soluble protein content and primary root length (Figure 4) for both lead and copper treatments in artichoke seedlings. It was seen that root total soluble protein content significantly affected root dry weight (R^2 = 0.9364 for lead and R^2 = 0.9094 for copper stress). In addition, primary root length was significantly affected by root total soluble protein content in artichoke seedlings exposed to lead and copper stresses (R^2 = 0.9189 for lead and R^2 = 0.9152 for copper stress).

The effect of Cu and Pb on genomes. Many studies examined the effects of several genotoxic agents on different plants and organisms by the use of RAPD markers (Liu et al. 2005, Cenkci et al. 2009, Cansaran et al. 2011). In some of these studies, genotoxic agents were implemented under control in a laboratory environment while some other studies examined the plants and organisms that were exposed to genotoxic agents in their natural habitats. For example, Liu et al. (2005) treated the seedlings of barley (*Hordeum vulgare* L.) with different concentrations of cadmium (Cd) and noted that RAPD is an efficient biomarker to determine the genotoxic effects of cadmium on plants. Moreover, they reported that the increase in the concentration of cadmium led to the increase in the polymorphism of the seedlings' RAPD profiles (Liu et al. 2005). In another study, Cenkci et al. (2009) treated the seedlings of bean (*Phaseolus vulgaris* L.) with different concentrations of mercury (Hg), boron (B), chrome (Cr) and zinc (Zn). These researchers examined the DNA samples from the roots and leaves by RAPD. At the end of this analysis, they determined the difference in the band patterns and increase in the polymorphism in the RAPD profiles of the plants that were treated with heavy metal compared to the control group (Cenkci et al. 2009). Cansaran et al. (2011) determined the effects of the pollution of air and heavy metal on lichen by identifying the variations in DNA bands by RAPD.



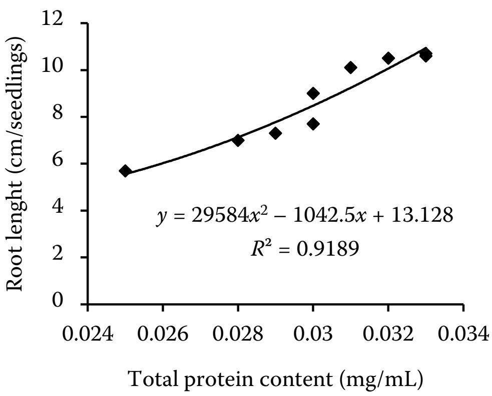


Figure 3. Influence of root total soluble protein content to root dry weight and length (Pb)

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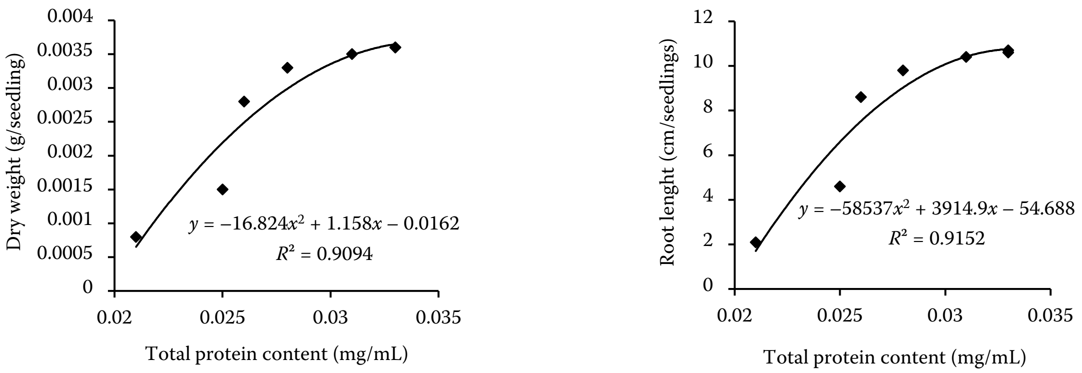
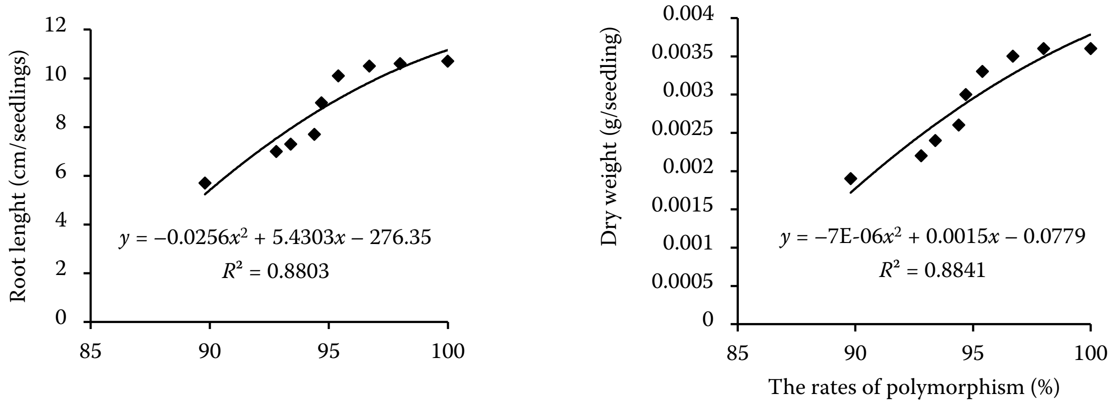


Figure 4. Influence of root total soluble protein content to root dry weight and length (Cu)

As a result, they noticed the relation between the accumulation of heavy metals in lichens and DNA polymorphism. They also expressed that RAPD is an efficient technique to determine the environmental toxicity level. Similarly, Soydam et al. (2012) exposed *Cucumis sativus* L. to the stress of the increasing concentrations of copper and zinc. At the end, they observed that the RAPD profiles of the samples that were treated with high concentrations of heavy metal solutions changed compared to the control group (Soydam et al. 2012). The researchers also noted that the reason for this change was the genotoxic and mutational effect of the increasing concentrations of heavy metals on plants.

In this study, in line with the previous findings in the literature, it was found that the treatment of increasing concentrations of lead and copper with the artichoke seedlings lead to the genotoxic effect on the genomes of the samples and polymorphism in the RAPD band profiles. 17 of 23 RAPD primers used in the study revealed polymorphic band formation that is different from the control group. OPC 11 (19.8%), OPC 06 (14.9%), and OPC 12 (14.3%) primers showed substantial polymorphic band patterns for the treated seedlings (Tables 3



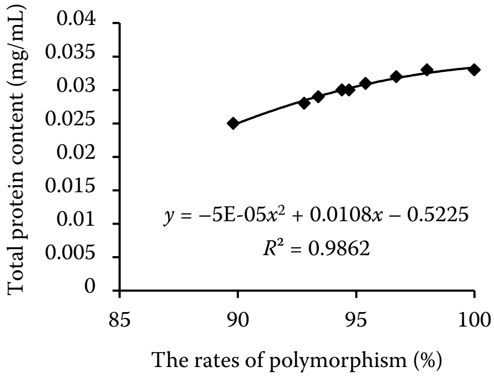


Figure 5. Influence of genomic template stability to the root length, dry weight of root and soluble protein content (Pb)

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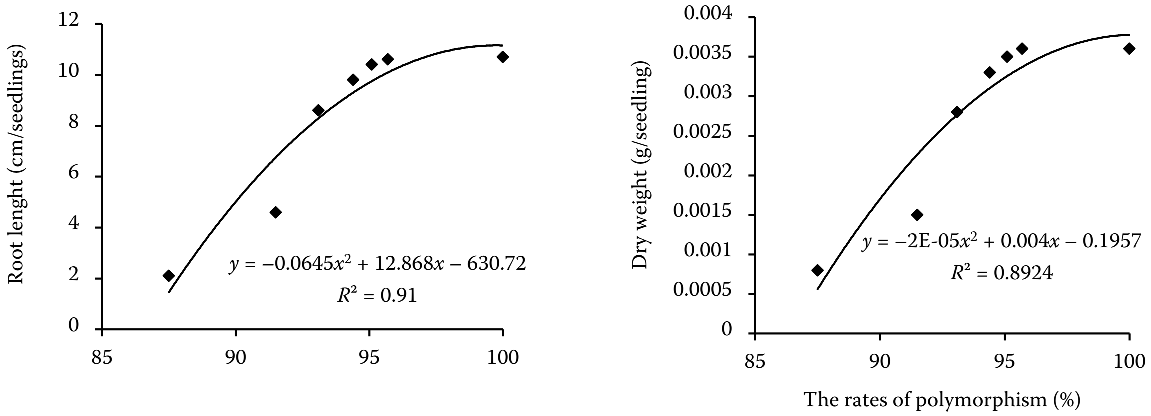

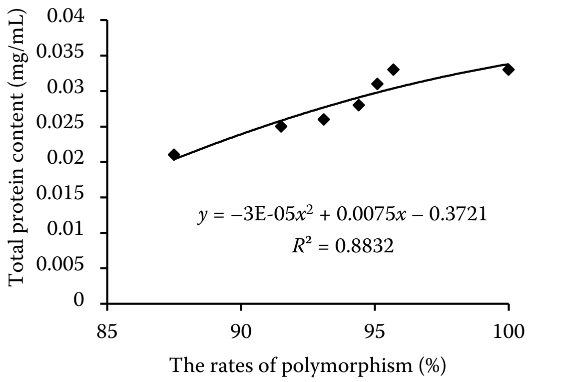

Figure 6. Influence of genomic template stability to the root length, dry weight of root and soluble protein content (Cu)

and 4). This situation showed that OPC 11, OPC 06, and OPC 12 primers are influential in revealing mutagenic effects of heavy metals for artichoke seedlings.

The polymorphism level in the samples of artichokes that were exposed to different concentrations of lead stress was between 2–9.2%, and it was between 4.3–12.5% in the samples of artichokes that were exposed to different concentrations of copper stress (Tables 3 and 4). Among all, the most polymorphism, which is 12.5%, was observed in the samples exposed to 320 ppm copper.

Comparison of the DNA changes with physiological and biochemical parameters. Genomic template stability (GTS, %) was used to compare the alteration in RAPD profiles with the decrease in root length, dry weight and total protein content in artichoke seedlings. The comparison of the sensitivity of GTS (%), root length, dry weight and total soluble protein content was calculated according to their control value which was set to 100% (Tables 3 and 4). GTS and root length (except 20 ppm for Cu and Pb), dry weight of root (except 20 ppm for Cu and Pb) and soluble protein content of root (except 20 ppm for Cu and Pb) values of artichoke seedlings, which was exposed to the increasing concentrations of Pb and Cu solutions, presented a decrease when compared to the control group seedlings (Tables 3 and 4). Also, it was determined that in the artichoke seedlings that were exposed to low concentrations of heavy metals, compared to the parameters of root length, dry weight of root and total soluble protein content, the GTS showed more changes (Figures 1 and 2). In addition, it was seen that the genomic template stability significantly affected primary root length (R^2 = 0.8803 and R^2 = 0.91 for lead and copper, respectively), root dry weight (R^2 = 0.8841 and R^2 = 0.8924 for lead and copper, respectively) and root total soluble protein content (R^2 = 0.9862 and R^2 = 0.8832 for lead and copper, respectively) (Figures 5 and 6).

In conclusion, it was noticed in this study that hyperaccumulator artichoke plants have physiological, biochemical and genomic traits associated with accumulation of lead and copper, which causes heavy metal stress, and it can be useful for restoring lead and copper contaminated sites up to a certain level. Also, our results indicate that artichoke has physiological traits associated with accumulation of lead up to a relatively high levels and it can be useful for restoring the lead-contaminated sites.

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