Original article

Potential of S-containing and P-containing complexones in improving phytoextraction of mercury by *Trifolium repens L.*

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**Abstract**

Mercury is a global pollutant in the modern world. There is a large number of areas in the world where mercury is present in soils in significant quantities. Remediation methods which have traditionally been proposed may pose a risk of secondary mercury contamination and/or adverse health effects for cleaners. Phytoextraction of heavy metals from the soil environment is currently considered one of the promising non-invasive methods of remediation. But this approach has limited effectiveness. Chemically induced phytoextraction can increase the efficiency of this process both by converting less bioavailable mercury compounds to bioavailable fractions in the soil and by increasing the rate of transfer of metals in plants. This paper presents the results of a screening study of various chemical amendments to enhance the phytoextraction of mercury by *Trifolium repens L.* The results showed good potential for the induction of phytoextraction of phosphorus (P) and sulfur (S)-containing chelates. With this study, for the first time for the phytoextraction of mercury, the monoethanolamine salt of 2,2′-(ethylenedithio) diacetic acid was used as the S-containing chelate, and the disubstituted potassium salt of 1-hydroxy ethylidene-1,1-diphosphonic acid was used as the P-containing chelate. Further attention is given to study the effect that exogenous application of phytohormones and plant growth regulators has on the efficiency of mercury absorption and physiological status of plants, which performed well in combination with a P-containing chelate.

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**1. Introduction**

Regeneration of the soil horizon is a complex task that has been the focus of numerous studies (Wang et al., 2017). Phytoextraction using plants to absorb heavy metals from the soil is currently considered one of the promising noninvasive methods of in situ remediation, which is widely used (Robinson et al., 2006) and has a great industrial and commercial potential (Chaney et al. 2007) (see Fig. 1).

Mercury is one of the most dangerous pollutants in the environment, and mercury-contaminated soils are one of the world’s major problems (Liu et al., 2020). As a result of various activities of industrial enterprises, such as metallurgical plants, production of mercury cells, chlorine/alkali production, incinerators, and other stationary sources of pollution, (Tarasova et al. 2017) large quantities of mercury enter in the environment, primarily in water bodies and soil (Kocman et al. 2013). The high toxicity, complex dynamics of the behavior of mercury in the environment (Ranieri et al., 2020), and the biomagnification trend in ecosystems warrant the classification of this chemical as a global pollutant (Makarova et al., 2020).

At the present, studies of chelate-assisted phytoextraction are widely developed, where chelating agents are used to increase absorption and accelerate the process (Fedotov et al., 2012) of cleaning contaminated soils (Meers et al., 2008). In the works (Evangelou et al., 2007b), extensive reviews of effects, mechanisms, toxicity and behavior in soils of various "agents for induced phytoextraction" were made. Organic complexing compounds of
the complexone class (Yoshikawa et al., 2001), which are capable of forming strong water-soluble complexes with metal ions, have become widespread as the latest agents (Khalid et al., 2013; Tsirulnikova et al., 2016). Thus far, successful results of induced phytoextraction have been obtained for many heavy metals (Cd, Ni, Zn, Cu, Pb, As, etc.). However, research and the search for effective plants and chemical corrections for phytoextraction of mercury are still underway (Ranieri et al., 2020). The peculiarities of the chemical nature of mercury and its behavior in the soil–plant system seriously complicate the work in this direction. First, mercury belongs to metals which are not essential to live organisms and plants, in contrast to several biometals and trace elements (Liu et al., 2018; Wang et al., 2018). Second, the group of plants capable of accumulating the element is still extremely small (Liu et al., 2018; Wang et al., 2018). Third, in the most plants, weak translocation of mercury to terrestrial organs (shoots, stems, and leaves) is observed, while the main amount of the absorbing element is deposited in the roots (Marrugo-Negrete et al., 2016; Rodriguez et al., 2007). Several foreign scientists have concentrated their efforts in the study and search for auxiliary reagents for phytoextraction of mercury (Qian et al., 2018). In this case, two approaches were used in the selection of inductors:

1) Mercury, which belongs to the zinc group of d-metals, has a high ability to form complexes, which is characteristic of d-metals. All elements of this group have a high affinity for the sulfur atom (Cassina et al., 2012), which significantly decreases in the series Hg > Cd > Zn. Mercury (II) is a soft Lewis acid and complexes readily with soft Lewis bases such as reduced-S ligands (Bower et al., 2008). The affinity for the donor sulfur atom is especially high in mercury (Moreno et al., 2005). This property served as a basis for the study of thiosulfates and halides as ligands for phytoextraction of mercury by foreign scientists. (Wang et al. 2011, 2018). Compounds containing sulfur or iodine atoms (Smolinska et al., 2015), such as ammonium thiosulfate (Wang et al., 2017), sodium thiosulfate, and potassium iodide (Smolinska et al., 2012), can act as ligands for mercury (Wang et al. 2012a).

2) Scientists have also suggested using well-known and common chelating agents such as complexones or low molecular weight organic acids (Grifoni et al., 2017). Published data include studies of various aminopolycarboxylic acids (Tandy et al., 2004), most notably ethylenediaminetetraacetic acid (EDTA) (Smolinska et al., 2012) ethylenediamine diisuccinic acid (Evangelou et al., 2007a), and nitrolotriacetic acid (Lomonte et al., 2011), and natural low molecular weight acids: citric (Smolinska et al., 2015), oxalic (Parra et al., 2008), etc.). Several authors also note the good efficiency of the ethylenediaminetetraacetic acid chelator (Smolinska et al., 2007), the disadvantage of which is the increased ability to leach other heavy metal ions from the soil and, thus, simultaneously increase the secondary pollution of soil and groundwater (Smolinska et al., 2015; Evangelou et al., 2007b).

An increase in the efficiency of induced phytoextraction is closely associated with the search for new formulas of chelators (Masoudi et al., 2020) and their successful combinations with other functional amendments, for example, with plant growth regulators (PGRs) (Sun et al., 2020). In the case of phytoextraction of mercury, it can be a derivative of a carboxyl-containing chelator with a sulfur atom as a coordination partner (S-containing chelate). According to the “hard and soft acid-base” theory, Hg preferentially forms complexes with soft ligands such as sulfur to form insoluble and stable compounds (Wang et al., 2020). In earlier studies, it was found that S-containing chelate is capable of forming fairly stable complexes with metal ions exhibiting an affinity for the sulfur atom, in particular, with the mercury (II) cation. In this regard, these compounds can be proposed for testing the possibility of their use in the process of phytoextraction of mercury (II). The results of studies related to the determination of the physicochemical properties, kinetics, and mechanism of interaction with cations, depending on the structure, opened up great opportunities for their application; interest in this class of compounds has not waned thus far (Tsirulnikova et al., 2020). Also, it can be a derivative of a phosphorus-containing complexone from the class of bisphosphonates (P-containing chelate), known for its biological activity, ability to form water-soluble complexes with mercury,

![Fig. 1. The study of obtaining the 2,2'--(ethylenedithio)diacetic acid.](image-url)
and less phytotoxicity compared to EDTA. Experimental studies with none of the above reagents have been carried out.

According to the reports written by several authors, previously published supplementation of chelate-assisted phytoextraction methods with treatment with PGRs showed a significant improvement in the overall process of phytoextraction of Pb (Hadi et al., 2010). It has been shown that the use of plant growth stimulating substances improves phytoextraction by increasing the growth of shoots and roots and, as a consequence, increasing the biomass yield in general (Liphadzi et al., 2006; Israr et al., 2011). Besides, exogenous phytohormones increase the effectiveness of plant antioxidant systems, thereby helping to reduce metabolic stress caused by high concentrations of heavy metals. For example, auxins are involved in cell division and elongation, growth and differentiation of organs, while gibberellins take part in seed germination, stem elongation, leaf expansion, etc. (Bulak et al., 2014). Auxins have an important effect on tropisms and are directly involved in the absorption and movement of cations (Vamerali et al., 2011). Gibberellins protect the photosynthetic apparatus of plants from the toxic effects of heavy metals. A detailed review by (Wang et al., 2012b) provides data on numerous aspects of the toxicity of mercury to plants, including inhibition of the antioxidant system and photosynthetic activity; inhibition of plant growth and assimilation of nutrients, homeostasis; induction of oxidative stress, etc. Given this circumstance, the combination of treatments with chelate and exogenous growth-regulating substances in the course of phytoextraction of mercury seems to be very expedient. Moreover, an additional factor that stabilizes the photosynthesis of a phytoextractor plant may be treated with iron chelate along with PGRs, which can make a certain contribution to the overall increase in biomass.

Thus, the stated arguments allowed the authors of this work to formulate the following research objectives:

- testing two new formulas of compounds as inducers of phytoextraction of mercury: S-containing chelate, and P-containing chelate. These chemicals are compared with EDTA and sodium thiosulfate;
- evaluating the combined use of a chelator and PGRs. Separately, assessing this complex with additional treatment with iron chelate.

As the S-containing chelate, it was proposed to use the monoethanolamine salt of 2,2’-(ethylenedithio) diacetic acid (MEDBA), the compound was specially synthesized for the experiment. In Russia, with MEDBA, vegetation and field experiments were carried out on some crops (potatoes, grapes, beets, leafy vegetables), in which MEDBA was used as a biological amendment that contributes to the development and increase of yields (Starovoitova et al., 2019). It should also be noted that MEDBA was previously proposed for the spectrophotometric determination of mercury (II), which is not interfered with by 100-fold amounts of Al(III), Ni(II), Cu(II), Pb(II), Mn(II), Mg(II), Zn(II), and Fe(III), MEDBA reacts with mercury (II) even in a strongly acidic medium (Khalid et al., 2013). At pH > 3, a mercury hydroxo complex formed.

It was proposed to investigate the potassium salt of 1-hydroxy ethylidene-1,1-diphosphonic (K₂HEDP) as a P-containing chelate. This compound is not a complex and in solution freely dissociates into a positively charged potassium ion and an anionic residue, which enters into complexation reactions with cations of other metals to form stable complexes.

Evaluation of the effectiveness of combining the methods of chelate and PGRs assisted phytoextraction was carried out on the example of complex processing of experimental samples with a P-containing chelate in combination with auxins, gibberellins, and iron chelate. Sodium salt of gibberellic acid and 4 (indole-3yl) butyric acid, as well as the sodium salt of ethylenediamine-N, N’-bis(hydroxyphenyl) acetic acid of iron (Na₂FeEDDHA) were used as PGRs and iron chelators, respectively.

2. Materials and methods

2.1. Research objects

In this study, experiments were carried out on white creeping clover (Latin Trifolium repens L.) seedlings. Trifolium repens L. is a key species in temperate meadow systems with high biomass, strong hardiness and adaptability to various environmental conditions. On the territory of the Russian Federation, this is a widespread wild-growing crop. Trifolium repens L. belongs to leguminous herbaceous crops. It is kept in the herbage for 2–3 years. The root system of Trifolium repens L. stems, with strongly branching lateral shoots, is located in the soil layer 40–50 cm. Trifolium repens L. and its varieties are not demanding on soils. It develops well on clay; loamy and sandy types have high winter and frost resistance. In research, Liu et al. (2018) showed that clover can develop in soil with multimetallic contamination and become the dominant species. And in the works of Kudryashova (2003) and Tribis (2016), when studying phytoextraction, the high efficiency of clover in the absorption of nickel, zinc, copper was noted.

2.2. Experiment description

Model experiments were carried out following the ISO 22030: 2005 standard “Soil quality. Biological methods. Chronic toxicity in higher plants”. Standard laboratory equipment, a phytolamp, a balance with an accuracy of ±0.1 mg, universal soil (pH 5.8–6.2), and a set of plastic vegetation pots for planting seeds with a volume of 1 litter were used in the experiments. The experiment was carried out in a laboratory located in Moscow from July 20 to August 17. The temperature during the experiment was 20–24 °C. Since the experiment was carried out in the summer months, no additional lighting was applied.

During the experiment, the vegetation pot was filled with universal soil with the addition of 237 mg of fertilizer containing 21% nitrogen, 11% phosphorus (P₂O₅), and 11% potassium. To simulate mercury pollution, an aqueous solution of Hg(NO₃)₂·H₂O was used. Hg(NO₃)₂·H₂O was added to the pots in amounts of 9.87 mg (5.77 mg Hg) or 19.73 mg (11.55 mg Hg). As a control, a vegetation pot with universal soil and fertilizer without the addition of mercury was used. In each vegetation pot, including the control vessel, 20 Trifolium repens L. seeds were planted. To assess the effectiveness of the impact of amendments on the degree of phytoextraction of mercury by Trifolium repens L. seedlings, the following substances were added to individual vegetation pots:

- Na₂EDTA: a weighed portion of 12.06 g was diluted in 600 ml of distilled water, 20 ml of solution was pipetted and added to pots from days 22 to 26 after planting.
- Sodium thiosulfate: a weighed portion of 6.6 g was diluted in 500 ml of distilled water and added from days 26 to 30 after planting at an amount of 17 ml per vegetation pot.
- MEDBA: 500 ml of 20% MEDBA solution was brought to 550 ml with distilled water and added from days 26 to 30 after planting at an amount of 18 ml per vegetation pot.
- K₂HEDP: 2 ml of 28.3% solution was diluted in 1 L of distilled water and added from days 22 to 26 after planting at an amount of 11 ml per vegetation pot.
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- Na(FeEDDHA): 1 ml of 0.1% solution was added in 1 L of distilled water was added on days 12, 20, and 28 after planting the seeds. The application was carried out by spraying on the plants in the second half of the day until drops appeared on the surface of the plant leaves.

- PGRs: sodium salts of gibberellic acid were added in the form of the “Zavyaz” preparation (SELHOZEKOSERVICE LLC, Russian Federation): 200 mg of the preparation was diluted in 1 L of distilled water and sprayed on the plants in the morning on days 12, 20, and 28 after planting. Spraying was carried out until drops appeared on the surface of plant leaves. 4 (indole-3yl) butyric acid was added in the form of “Kornevin” preparation (“Orton” LLC, Russian Federation): 0.7 g of the preparation was diluted in 1 L of distilled water. Then, 10 ml of the resulting solution was added to each vegetation pot using a pipette.

Additionally, for comparison, vegetation pots were prepared with mercury-contaminated soil, in which no amendments were provided. All variants of vegetative pots with amendments, with mercury but without amendments, and controls with clean soil were prepared in triplicate.

The plants were removed 33 days after planting the seeds. The removed Trifolium repens L. seedlings were cleared of soil, washed with water, and divided into the shoot and root parts. Then, the shoots and roots were dried, and the mass of the obtained samples was measured. Seedling weight measurements were carried out to assess the effect of mercury and amendments on plant growth and development.

2.3. Analytical methods.

In the obtained samples of shoots and roots, 58 elements were determined (Li, Be, B, Na, Mg, Al, P, S, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Mo, Rh, Ag, Pd, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Ir, Pt, Au, Tl, Pb, Bi, Th, and U). The determination method is based on the use of autoclave (with resistive heating) acid decomposition of the analyzed samples and subsequent analysis of the resulting solution by two multielement methods: atomic emission with inductively coupled plasma (ICP-AES) and mass spectrometry with inductively coupled plasma (ICP-MS). The ICP-AES method (iCAP-6500, Thermo Scientific, USA) allowed us to determine the contents of Li, B, Na, Mg, Al, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Mo, Rh, Ag, Pd, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Ir, Pt, Au, Tl, Pb, Bi, Th, and U. The determination method was carried out with the following operating parameters of the spectrometer: generator output power – 1200 W; reflected power < 5 W; spray chamber type concentric; the flow rate of the plasma-forming flow Ar – 13 l/min; auxiliary flow Ar – 0.8 l/min; Ar flow rate in the nebulizer – 0.8 l/min; the flow rate of the analyzed sample is 1.5 ml/min. Definition of the content of elements in aqueous solutions was carried out by a quantitative method using standard solutions containing 0.5 and 10 mg/L of the studied elements. The relative standard deviation for all elements did not exceed 0.2 when measuring the content of these elements up to 5× detection limit (DL) and did not exceed 0.15 when measuring the content > 5× DL.

The soil remaining after excavation was dried, its mass and mercury content were measured. To determine the mercury content in the dried soil samples, portions of the analyzed samples weighing 200 mg were placed in Teflon cups, wetted with a mixture of hydrochloric and nitric acids 3:1, and boiled for 5 min; then, 5–10 cm³ of water was added for laboratory analysis. The resulting solutions were transferred into polyethylene bottles, diluted with water for laboratory analysis to 20 cm³, and left for 1 h to precipitate suspended particles of the undissolved part of the analyzed samples, after this procedure the obtained solutions were analyzed. Determination of mercury in the obtained solutions was carried out by inductively coupled plasma mass spectrometry (X-7, Thermo Elemental, USA).

2.4. Obtaining MEDBA

The most common method for producing 2,2’-(ethylenedithio)diacetic acid is the nucleophilic substitution of chlorine in dichloroethane by interaction with thiglycolic acid in an alkaline medium and isolation of the end-product by acidification of the reaction solution with mineral acid. The reaction yield is approximately 70% (Proshaskova et al., 1973). Despite the simplicity of the method, its disadvantage is the need to use toxic and unstable thiglycolic acids in the reaction.

The existing disadvantage of the synthetic chemistry of complexones containing thiglycolic groups was eliminated in the 1960s–1970s. It was found that the condensation of thiourea with monochloroacetic acid (or its sodium salt) ends with the formation of amidinothioacetic acid or its cyclic analog, iminohiazolidone (Sen Gupta Kalyan et al., 1977). Both products are odorless white substances that are practically insoluble in water, with an unlimited retention period. In an alkaline medium, amidinothioacetic acid and iminohiazolidone are quantitatively decomposed with the formation of the sodium salt of thiglycolic acid, gaseous ammonia and carbon dioxide (Sunjevic et al., 1969).

Further development of the chemistry of S-containing chelate was devoted to studies to improve the synthesis of known chelates, to obtain a series of new compounds, to clarify the possibilities of their use, and to study the reactivity of thiglycolic acid in nucleophilic substitution reactions (Sunjevic et al., 1969).

The quantitative formation of thiglycolic acid in the process of hydrolysis in an alkaline medium was the basis for using amidinothioacetic acid and iminohiazolidone as starting materials for obtaining 2,2’-(ethylenedithio)diacetic acid according to the scheme presented below. In the reaction of nucleophilic substitution of chlorine is interacting with dichloroethane in an alkaline medium and isolating the target product upon acidification of the reaction mixture with mineral acid (e.g. HCl).

The study of obtaining the 2,2’-(ethylenedithio)diacetic acid process made it possible to establish the optimal conditions for its implementation to obtain the end product with a yield of 86% (70%) according to the developed technological scheme, having implemented it in industrial conditions. Note that 2,2’-(ethylenedithio)diacetic acid is practically insoluble in water, and therefore, the use of the reagent presupposes its preliminary transformation into a soluble state. With this aim, disubstituted 2,2’-(ethylenedithio)diacetic acid salt with monoethanolamine (MEDBA), which is a plant growth stimulator, was proposed for the first time (Tsirulnikova et al., 2016).
3. Results and discussions

3.1. Screening of chelating amendments

It should be noted that visually, on the 33rd day of the experiment (after planting the seeds), the *Trifolium repens* L. seedlings in vegetation pots with clean soil, soil contaminated with mercury and soil contaminated with mercury treated with sodium thiosulfate and K₂HEDP (Fig. 2a) looked approximately the same: most of the plants were green, but some of them had whitish leaves. Plants in vegetation pots with mercury-contaminated soil and Na₂EDTA-treated plants looked slightly wilted, with some leaves showing signs of necrosis. Plants in vegetation pots with the addition of MEDBA (Fig. 2b) looked the weakest: most of the seedlings died (wilted), some leaves were yellow, and an oily bloom was visible on the soil.

The results of the measured data for the experimental samples described above are shown in Table 1. This table shows that the largest mass of both roots and shoots was found for the *Trifolium repens* L. seedlings grown on clean soil followed by seedlings grown on soil contaminated with mercury. And a high concentration of mercury had a more pronounced negative effect on the mass of seedling organs.

The dependencies of the average *Trifolium repens* L. seedlings growth on the degree of soil pollution and the introduction of various amendments are shown in Fig. 3.

Fig. 3 illustrates a significant increase in the negative effect of mercury when it was introduced at an amount of 11.55 mg Hg per vegetation pot compared to 5.77 mg Hg per vegetation pot. When 11.55 mg Hg per vegetation pot is applied, the growth and development of plants grown on soil contaminated with mercury lag behind the *Trifolium repens* L. seedlings grown on pure soil, while when mercury is introduced into the soil at a lower dose (5.77 mg Hg per vegetation pot), this effect is not observed. It is also possible to note the inhibitory effect of MEDBA on plant development in the concentration proposed by the authors in comparison with other amendments.

Fig. 4 shows the values for the bioconcentration factor (BAF) of mercury for the *Trifolium repens* L. seedlings shoots (BAFshoots), roots (BAFroots), and the plant as a whole (BAFtotal), depending on the amendments used.

BAFshoots and BAFroots were defined as the ratio of the concentration of mercury in shoots or roots to the concentration of mercury in the soil. BAFtotal was calculated using the following formula (Cojocaru et al., 2016):

$$BAF_{total} = \frac{C_{Hg, shoots} m_{Hg, shoots} + C_{Hg, roots} m_{Hg, roots}}{C_{Hg, soil}}$$

where $C_{Hg, shoots}$ - concentration of mercury in plant shoots; $m_{Hg, shoots}$ - shoots mass; $C_{Hg, roots}$ - concentration of mercury in plant roots; $m_{Hg, roots}$ - roots mass; $C_{Hg, soil}$ - concentration of mercury in soil.

The graphs presented in Fig. 4 clearly show that the BAF for most amendments depends on the concentration of mercury in the soil. The higher the concentration is, the higher the BAF; the exception is K₂HEDP, for which the dependence is inverse.

Fig. 4 also shows that despite the introduction of MEDBA at increased doses, which had harmed the growth and development of shoots, the results obtained allowed us to record a positive effect of MEDBA in comparison with thiosulfate and K₂HEDP, in some cases also with Na₂EDTA. At the same time, BAFtotal for MEDBA was the best among all tested amendments with values 3.65 and 6.38 for soil with 5.77 mg Hg and 11.55 mg per vegetation pot, respectively. It should be noted that for clean soil with a Hg concentration of 2.8 μg/g soil, BAFshoots, Mercury is significantly higher than that observed in contaminated soil cases.

Fig. 5 shows the results of a comparative assessment of the translocation factor (TF) of mercury from roots to shoots for the *Trifolium repens* L. seedlings, defined as the ratio of the concentration of a substance in the shoot to the concentration in the roots when using different amendments.

Fig. 5 shows that the highest TF value for soil with 5.77 mg Hg per vegetation pot is for MEDBA. For soil with 11.55 mg Hg per vegetation pot, the highest TF is found for Na₂EDTA, which is comparable to the results for MEDBA. However, the highest TF is observed for clean soils.

3.2. The effectiveness of additional treatments with PGRs and iron chelate

Taking K₂HEDP as an example, this work also tested the effect of PGRs, such as sodium salt of gibberellic acid and 4 (indole-3yl) butyric acid, in combination with iron chelate Na (FeEDDHA) on the absorption of mercury by the *Trifolium repens* L. seedlings. Table 2 shows the results of the K₂HEDP experiment with and without PGRs and Na (FeEDDHA). From the data shown in the table, it can be seen that using PGRs and Na (FeEDDHA) has a positive effect on the *Trifolium repens* L. seedlings biomass, and the greatest effect is achieved when PGRs and Na(FeEDDHA) are used together.

With mercury contamination of 5.77 mg, the average increase in the *Trifolium repens* L. seedlings biomass with additional treatments with PGRs was 16.9% compared to treatment with only one chelating agent (K₂HEDP). Whereas the inclusion of additional treatment with Na (FeEDDHA) led to an even greater significant increase in biomass up to 35.6%. At higher mercury concentrations (11.55 mg Hg per 1 vegetation pot), additional phytohormone corrections also led to an increase in biomass, but less: 5.4% when treated with PGRs and 27% when treated together with iron chelate (PGRs + Na (FeEDDHA)). Such an increase in biomass, combined with an increase in the concentration of mercury in the shoots and roots of plants, made it possible to record an overall increase in the absorption of mercury by the *Trifolium repens* L. seedlings by 69% and 74.5% with the initial contamination of mercury of 5.77 mg Hg and 11.55 mg Hg per 1 vegetation pots, respectively.

A similar conclusion can be drawn from Fig. 6. Additional PGRs and Na (FeEDDHA) have a stimulating effect on the *Trifolium repens* L. seedlings’ growth. At the same time, in the case of a high content of mercury (when 11.55 mg Hg was added per vegetation pot), even additional PGRs and Na (FeEDDHA) are not enough for the *Trifolium repens* L. seedlings to catch up in growth and development compared to those growing on clean soil. The toxicity of mercury was reduced by using PGRs and Na (FeEDDHA).

Fig. 7 shows that the treatment of seedlings with PGRs and Na (FeEDDHA) positively influenced not only the growth and development of the *Trifolium repens* L. seedlings but also the degree of absorption of mercury by both roots and shoots of the *Trifolium repens* L. seedlings. It should be noted that the degree of absorption of mercury using P-containing chelate (K₂HEDP) together with PGRs and Na (FeEDDHA) in some cases becomes comparable to S-containing chelate (MEDBA).

At low concentration of mercury (5.77 mg Hg per 1 vegetation pot), the use of the PGRs + Na (FeEDDHA) stimulated the accumulation of mercury in the roots (see Fig. 8): TF – 0.07, and at higher concentration much better translocation was observed in ground bodies and TF amounted to — 0.37. This TF value was the best one obtained in this experiment.

3.3. Analysis of the elemental composition of plant organs

The results of the conducted elemental composition of the *Trifolium repens* L. seedlings are presented in Appendix 1. The content
Fig. 2. The *Trifolium repens* L. seedlings in vegetation pots on the 33rd day of the experiment (after planting the seeds) with soil with 5.77 mg Hg (left row on photo) and 11.55 mg Hg (right row on photo) per vegetation pot with a) K₂HEDP; b) MEDBA.

Table 1
Results of a laboratory experiment on mercury's phytoextraction of the *Trifolium repens* L. seedlings in the presence of various amendments.

| Measured parameters       | Number of seedlings, pcs | Plant weight, g | Hg concentration, μg/g |  |
|---------------------------|--------------------------|-----------------|------------------------|---|
|                           |                          | Shoots          | Roots                  |  |
| Clean soil                | 9                        | 0.1463          | 0.0127                 | 2.8 ± 3.9 | 4.1 ± 0.15 | 3.4 ± 0.15 |
| 5.77 mg Hg                | 12                       | 0.0603          | 0.0043                 | 19.9 ± 8.4 | NBD        | NBD        |
| 5.77 mg Hg + Na₂EDTA      | 11                       | 0.0507          | 0.0090                 | 38.5 ± 10.2 | 62.6 ± 0.15 | 453 ± 0.15 |
| 5.77 mg Hg + Sodium thiosulfate | 12          | 0.0643          | 0.0110                 | 83.1 ± 33.4 | 105 ± 0.15 | 883 ± 0.15 |
| 5.77 mg Hg + MEDBA        | 13                       | 0.0853          | 0.0083                 | 27.7 ± 15.4 | 85.2 ± 2.8  | 289 ± 0.15 |
| 5.77 mg Hg + K₂HEDP       | 11                       | 0.055           | 0.004                  | 25.8 ± 8.0  | 72.7 ± 22.4 | 336 ± 0.15 |
| 11.55 mg Hg               | 8                        | 0.0330          | 0.0057                 | 39.1 ± 12.6 | NBD        | NBD        |
| 11.55 mg Hg + Na₂EDTA     | 8                        | 0.0287          | 0.0050                 | 45.4 ± 18.3 | 194 ± 0.15 | 691 ± 0.15 |
| 11.55 mg Hg + Sodium thiosulfate | 10         | 0.0350          | 0.0030                 | 73.5 ± 25.6 | 133 ± 6    | 841 ± 0.15 |
| 11.55 mg Hg + MEDBA       | 11                       | 0.0333          | 0.0073                 | 48.5 ± 11.6 | 204 ± 0.15 | 788 ± 0.15 |
| 11.55 mg Hg + K₂HEDP      | 8                        | 0.032           | 0.005                  | 55.9 ± 21.5 | 100 ± 0.15 | 531 ± 0.15 |

NBD - The concentration has not been determined.

Fig. 3. Dependencies of the *Trifolium repens* L. seedlings growth on the degree of initial soil mercury contamination and the introduction of various amendments with a) 5.77 mg Hg per vegetation pot; b) 11.55 mg Hg per vegetation pot.
of elements in the *Trifolium repens* L. seedlings shoots when using various amendments and Appendix 2 'The content of elements in the *Trifolium repens* L. seedlings roots when using various amendments'. In general, it should be noted that the elemental composition of the *Trifolium repens* L. seedlings grown on soils with the addition of S-containing chelate (MEDBA) significantly differ from the elemental composition of the *Trifolium repens* L. seedlings grown on soils with other amendments. In the *Trifolium repens* L. seedlings shoots and roots, there was a significant decrease in the content of K (4–5 times) and a significant increase in the content of S (4–12 times), as well as reduced content of Li, Mg, Ca, P, Mn, Z, Rb. Also, in the *Trifolium repens* L. seedlings shoots which grown on soils with the addition of MEDBA, increased content of Al (2–4 times) and Tl (1.5–2 times), as well as reduced content of Cs and Zn, were observed.

### 4. Conclusions

As a result of the experimental studies, data were obtained on the efficiency of absorption of mercury by white creeping clover (*Trifolium repens* L.), considering the introduction of various chelating agents, including two new formulas: 1) S-containing chelate (MEDBA) and 2) P-containing chelate (K₂HEDP). Both compounds have shown the ability to enhance the absorption of mercury by creeping clover. The selected concentrations of MEDBA had a pronounced inhibitory effect on the growth and development of plants, even the death of seedlings was noted; therefore, in further experiments with this reagent, it is recommended to decrease the concentration and approach the level of application of classical carboxyl-containing complexones, for example, EDTA.

The experiments carried out on phytoextraction of mercury also showed a significant increase in efficiency when combining the methods of chelate and PGRs-assisted phytoextraction. The results obtained are in good agreement with the data of previous studies (Lopez et al., 2005; Hadi et al., 2010), which used a combination of EDTA, exogenous treatment with auxins and gibberellins, and confirm the positive effect of this technique in phytoextraction of mercury. Moreover, a significant contribution to the increase in biomass and total accumulation was made by additional treatment with iron chelate, which indirectly indicates additional stabilization of the photosynthetic activity of phytoextractor plants. Thus, the inclusion of additional treatments with iron chelate also deserves extended testing in heavy metal phytoextraction problems. It should be noted that despite the increase in plant biomass, the positive effect of the (PGRs + Na (FeEDDHA)) component on the translocation coefficient remains unclear and requires further research.

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**Fig. 4.** Dependencies of BAF for the *Trifolium repens* L. seedlings on the degree of soil contamination with mercury and the introduction of various amendments: a) BAFshoots, b) BAFroots, c) BAFtotal.

**Fig. 5.** Dependence of TF for the *Trifolium repens* L. seedlings on the degree of soil contamination with mercury and amendments.

**Table 2** Comparative results of a laboratory experiment for phytoextraction of mercury by the *Trifolium repens* L. seedlings with and without PGRs and Na (FeEDDHA).

| Measured parameters | Number of seedlings, pcs | Plant weight, g | Hg concentration, μg / g |
|---------------------|--------------------------|-----------------|--------------------------|
|                     |                          | Shoots          | Roots                    | Soil | Shoots | Roots |
| 5.77 mg Hg + K₂HEDP | 11                       | 0.055           | 0.004                    | 25.8 ± 8.0 | 72.7 ± 22.4 | 336 ± 0.15 |
| 5.77 mg Hg + K₂HEDP + PGRs | 11 | 0.064 | 0.005 | 31.5 ± 10.6 | NBD | NBD |
| 5.77 mg Hg + K₂HEDP + PGRs + Na(FeEDDHA) | 12 | 0.074 | 0.006 | 28.7 ± 5.0 | 57.6 ± 19.8 | 798 ± 0.15 |
| 11.55 mg Hg + K₂HEDP | 8                       | 0.032           | 0.009                    | 55.9 ± 21.5 | 100 ± 0.15 | 531 ± 0.15 |
| 11.55 mg Hg + K₂HEDP + PGRs | 11 | 0.036 | 0.003 | 66.8 ± 22.7 | NBD | NBD |
| 11.55 mg Hg + K₂HEDP + PGRs + Na(FeEDDHA) | 10 | 0.041 | 0.006 | 46.1 ± 10.2 | 178 ± 0.15 | 486 ± 0.15 |

NBD - The concentration has not been determined.

1. https://doi.org/10.17632/zmw98h8ggf.1

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It is also very promising to use MEDBA in combination with additional PGRs and Na (FeEDDHA). However, their positive effect on the translocation coefficient is still not fully understood and requires further research.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgments**

Funding: The work was funded by MUCTR according to the research project № 3-2020-039.

**Data statement**

As the data were unsuitable to post, survey respondents were assured that raw data would remain confidential and would not be shared.

Data not available or the data that have been used are confidential.
| Elements Clean soil | Na$_2$EDTA | Sodium thiosulfate | MEDBA | K$_2$HEDP | K$_2$HEDP + PGRs + Na (FeEDDHA) |
|--------------------|------------|--------------------|-------|-----------|---------------------------------|
| Li                 | 0.77       | 0.81               | 0.82  | 0.89      | 0.84                           |
| Be                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| B                  | 12.5       | 9.6                | 9.0   | 7.3       | 15.0                           |
| Na                 | 913        | 4968               | 5602  | 12,465    | 11,264                         |
| Mg                 | 3545       | 4901               | 5278  | 4327      | 3962                           |
| Al                 | 45         | 76.0               | 72.2  | 67.3      | 59.1                           |
| P                  | 7911       | 8218               | 7828  | 7909      | 8042                           |
| S                  | 4738       | 6348               | 6190  | 19,518    | 16,252                         |
| K                  | 66,230     | 52,946             | 52,657| 48,138    | 45,328                         |
| Ca                 | 22,035     | 19,973             | 16,808| 16,596    | 15,231                         |
| Sc                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Ti                 | 4.0        | 7.3                | 49.9  | 8.0       | 12.0                           |
| V                  | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Cr                 | 0.8        | < DL               | < DL  | < DL      | < DL                           |
| Mn                 | 122        | 180                | 192   | 116       | 121                           |
| Fe                  | 156        | 719               | 619   | 251       | 200                           |
| Co                 | 0.32       | 0.27               | 0.25  | 0.17      | 0.16                           |
| Ni                 | 6.4        | 0.87               | 1.16  | 0.77      | 0.81                           |
| Cu                 | 2.1        | 2.9                | 2.7   | 2.6       | 2.8                           |
| Zn                 | 65.2       | 90.9               | 84.8  | 61.3      | 61.6                           |
| Ga                 | < DL       | 0.058              | 0.038 | < DL      | 0.046                          |
| As                 | 0.18       | 0.164              | 0.108 | 0.076     | 0.081                         |
| Se                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Rb                 | 15.2       | 12.5               | 12.4  | 10.3      | 11.2                           |
| Sr                 | 46.2       | 37.9               | 32.4  | 31.8      | 28.7                           |
| Y                  | < DL       | 0.212              | 0.16  | 0.040     | 0.041                         |
| Mo                 | 0.58       | 0.61               | 2.03  | 0.57      | 0.66                           |
| Rh                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Pd                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Ag                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Cd                 | 0.19       | 0.12               | 0.113 | 0.083     | 0.045                         |
| Sn                 | < DL       | 0.12               | 0.19  | 0.16      | 0.11                          |
| Sb                 | < DL       | 0.071              | 0.067 | 0.051     | 0.052                         |
| Te                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Cs                 | < DL       | 0.031              | 0.030 | 0.031     | 0.027                         |
| Ba                 | 7.1        | 6.54               | 6.21  | 6.42      | 6.54                          |
| La                 | 0.047      | 0.072              | 0.091 | 0.072     | 0.053                         |
| Ce                 | 0.090      | 0.155              | 0.19  | 0.14      | 0.11                          |
| Pr                 | 0.007      | 0.019              | 0.021 | 0.015     | 0.012                         |
| Nd                 | 0.025      | 0.076              | 0.083 | 0.069     | 0.047                         |
| Sm                 | 0.005      | 0.015              | 0.017 | 0.0127    | 0.0092                       |
| Eu                 | < DL       | 0.003              | 0.003 | 0.003     | 0.002                         |
| Gd                 | 0.0069     | 0.016              | 0.02  | 0.01      | 0.01                          |
| Tb                 | < DL       | 0.003              | < DL  | < DL      | < DL                           |
| Dy                 | < DL       | 0.022              | 0.0221| 0.0076    | 0.0078                       |
| Ho                 | < DL       | 0.0056             | 0.0036| 0.0076    | 0.0078                       |
| Er                 | < DL       | 0.022              | 0.0189| 0.0045    | 0.0035                       |
| Tm                 | < DL       | 0.003              | 0.0022| 0.003     | 0.002                         |
| Yb                 | < DL       | 0.021              | 0.0166| 0.0040    | 0.0027                       |
| Lu                 | < DL       | 0.0031             | < DL  | < DL      | < DL                           |
| Re                 | < DL       | 0.0051             | 0.0047| 0.0053    | 0.0041                       |
| Ir                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Pt                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Au                 | < DL       | < DL               | < DL  | < DL      | < DL                           |
| Tl                 | 0.033      | 0.074              | 0.082 | 0.063     | 0.059                         |
### Appendix 2

| Elements Clean soil | Na₂EDTA | Sodium thiosulfate | MEDBA | K₂HEDP | K₂HEDP + PGRs + Na(FeEDDHA) |
|---------------------|---------|---------------------|-------|--------|-----------------------------|
|                     | Hg      | Hg                  | Hg    | Hg     | Hg                          |
| Pb                  | 0.38    | 0.91                | 1.22  | 0.51   | 0.47                        |
| Bi                  | < DL    | 0.020               | 0.008 | 0.009  | < DL                        |
| Th                  | < DL    | 0.010               | 0.013 | 0.016  | 0.010                       |
| U                   | 0.010   | 0.021               | 0.018 | 0.021  | 0.018                       |
|                    |         |                     |       |        |                             |
| Li                  | 0.16    | 0.21                | 0.34  | 0.38   | 0.17                        |
| Be                  | < DL    | < DL                | 0.019 | 0.032  | < DL                        |
| B                   | 12.5    | 11.3                | 12.2  | 12.0   | 9.0                         |
| Na                  | 284     | 1094                | 2473  | 4203   | 1658                        |
| Mg                  | 1988    | 2471                | 2864  | 2193   | 1296                        |
| Al                  | 247     | 453.5               | 642.8 | 819.0  | 303.5                       |
| P                   | 6540    | 8688                | 9097  | 6569   | 2427                        |
| S                   | 4731    | 5837                | 7188  | 14,746 | 85,561                      |
| K                   | 31,669  | 24,953              | 35,521| 24,833 | 6098                        |
| Ca                  | 6683    | 9037                | 13,287| 12,650 | 11,699                      |
| Sc                  | < DL    | < DL                | < DL  | < DL   | < DL                        |
| Ti                  | 12.0    | 52.2                | 41.2  | 60.4   | 19.2                        |
| V                   | < DL    | < DL                | 1.3   | 1.1    | 1.2                         |
| Cr                  | 1.0     | 3.2                 | 1.6   | 1.6    | 1.2                         |
| Mn                  | 62      | 108                 | 187   | 284    | 26.8                        |
| Fe                  | 305     | 547                 | 954   | 740    | 338                         |
| Co                  | 0.78    | 0.80                | 0.98  | 1.4    | 0.27                        |
| Ni                  | 25.3    | 2.1                 | 2.9   | 2.0    | 0.9                         |
| Cu                  | 5.6     | 8.3                 | 6.5   | 4.5    | 4.3                         |
| Zn                  | 71.7    | 33.3                | 43.7  | 44.6   | 20.8                        |
| Ga                  | 0.085   | 0.109               | 0.165 | 0.189  | 0.067                       |
| As                  | 0.017   | 0.925               | 0.603 | 0.979  | 0.153                       |
| Se                  | < DL    | < DL                | < DL  | < DL   | < DL                        |
| Rb                  | 6.6     | 6.2                 | 8.7   | 6.7    | 1.8                         |
| Sr                  | 21.1    | 27.0                | 31.1  | 29.1   | 28.7                        |
| Y                   | 0.010   | 0.25                | 0.51  | 0.40   | 0.19                        |
| Mo                  | 2.1     | 1.7                 | 1.64  | 1.25   | 0.87                        |
| Rh                  | < DL    | < DL                | < DL  | < DL   | < DL                        |
| Pd                  | < DL    | < DL                | < DL  | < DL   | < DL                        |
| Ag                  | 0.06    | 0.34                | 0.32  | 0.09   | 0.16                        |
| Cd                  | 1.4     | 0.43                | 0.51  | 0.69   | 0.16                        |
| Sn                  | < DL    | 1.06                | 0.40  | 0.14   | 0.23                        |
| Sb                  | < DL    | < DL                | 0.159 | < DL   | < DL                        |
| Te                  | < DL    | < DL                | < DL  | < DL   | < DL                        |
| Cs                  | 0.018   | 0.038               | 0.054 | 0.061  | 0.025                       |
| Ba                  | 6.9     | 10.52               | 12.81 | 13.56  | 9.71                        |
| La                  | 0.23    | 0.36                | 0.53  | 0.54   | 0.24                        |
| Ce                  | 0.40    | 0.71                | 1.08  | 1.03   | 0.48                        |
| Pr                  | 0.034   | 0.079               | 0.123 | 0.12   | 0.054                       |
| Nd                  | 0.13    | 0.326               | 0.498 | 0.46   | 0.215                       |
| Sm                  | 0.026   | 0.058               | 0.099 | 0.10   | 0.038                       |
| Eu                  | 0.006   | 0.015               | 0.022 | 0.022  | 0.011                       |
References

Bower, J., Savage, K.S., Weisman, B., Barnett, M.O., Hamilton, W.P., Harpfer, W.F., 2008. Immobilization of mercury by pyrite (FeS₂). Environ. Pollut. 156 (2), 504–514. https://doi.org/10.1016/j.envpol.2008.01.011.

Bulak, P., Walkiewicz, A., Brzezinska, M., 2014. Plant growth regulators-assisted phytoextraction. Biologia Plantarum. 58, 1–8. https://doi.org/10.1007/s10535-013-0382-5.

Cassina, L., Tassi, E., Pedron, F., Petruzelli, G., Paolo, A., Barbafieri, M., 2012. Using a plant hormone and a thiolagent to improve phytoextraction of Hg-contaminated soil from a potussic chemical plant. J Hazard Mater. 231–232, 36–42. https://doi.org/10.1016/j.jhazmat.2012.06.031.

Chaney, R.L., Angle, J.C., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.V., 2008. Immobilization of mercury by pyrite (FeS₂). Environ. Pollut. 156 (2), 504–514. https://doi.org/10.1016/j.envpol.2008.01.011.

Fedotov, P.S., Kördel, W., Miró, M., Peijnenburg, W.J.G.M., Wennrich, R., Huang, P.-C., 2012. Multi contaminant metal speciation in a multi-contaminated industrial soil. AIMS Environ. Sci. 4 (2), 187–205. https://doi.org/10.3934/ensci.2017.2.187.

Hadi, F., Bano, A., Fuller, M.P., 2010. The improved phytoextraction of lead (Pb) and the growth of maize (Zea mays L.): the role of plant growth regulators (GA₃ and IAA) and EDTA alone and in combinations. Chemosphere 80 (4), 457–462. https://doi.org/10.1016/j.chemosphere.2010.04.020.

Israr, M., Jewell, A., Kumar, D., Sahi, S.V., 2011. Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of S. drummondii. J. Hazard. Mater. 186 (2–3), 1520–1526. https://doi.org/10.1016/j.jhazmat.2010.12.021.

Khalid, F., Tahir, M., Fiaz, N., Nadeem, M.A., Gillani, S.M.W., 2013. Hybrid maize response to assisted chelated and no chelated foliar applied zinc rates. J. Agric. Techn. 9 (2), 295–309. http://www.jat-aatsea.com (accessed 13 January 2021).

Kocman, D., Horvat, M., Pirrone, N., Cinnirella, S., 2013. Contribution of contaminated sites to the global mercury budget. Environ. Res. 125, 160–170. https://doi.org/10.1016/j.envres.2012.12.011.

Kudryashova, V.I., 2003. Accumulation of heavy metals by wild plants. PhD Thesis of biological sciences. N.P.Ogarov Mordovia State University, Saransk, Russian Federation, 27 Nov 2003.

Lipphardi, M.S., Kirikm, M.B., Paulsen, G.M., 2006. Auxinenhanced root growth for phytoextraction of sewagesludge amended soil. Environ. Technol. 27 (6), 695–704. https://doi.org/10.1080/09593332708618683.

Liu, Z., Chen, B., Wang, L., Urbanovich, O., Nagorskaya, L., Xu, X., Tang, L., 2020. A review on phytoextraction of mercury contaminated soils. J. Hazard. Mater. 400, https://doi.org/10.1016/j.jhazmat.2020.123138 123138.

Liu, Z., Wang, L., Ding, S., Xiao, H., 2018. Enhancer assisted-phytoextraction of mercury-contaminated soils by Oenolus Corniculato L. and Rhizosphere microorganism distribution of Oenolus Corniculato L. Ecotoxicol. Environ. Saf. 160, 171–177. https://doi.org/10.1016/j.ecoenv.2018.05.041.

Lomonte, C., Coronado, A., Gregory, D., Baker, A.J.M., Kolev, S.D., 2011. Chelate-assisted phytoextraction of mercury in biosolids. Sci. Total Environ. 409 (13), 2685–2692. https://doi.org/10.1016/j.scitotenv.2011.03.037.

López, M.L., Peralta-Videa, J.R., Benitez, T., Gardea Torresley, J.L., 2005. Enhancement of lead uptake by alfalfa (Medicago sativa) using EDTA and a plant growth promoter. Chemosphere 61 (4), 595–598. https://doi.org/10.1016/j.chemosphere.2005.02.028.

Makarova, A., Yakubov, R., Varbanov, P.S., 2020. Global mercury footprint evaluation of new biodegradable chelants in enhancing phytoextraction of heavy metals from a contaminated calcareous soil. J. Environ. Health Sci. Eng. 18, 655–664. https://doi.org/10.1186/s40068-020-00491-y.

Meers, E., Tack, F.M.G., Van Slyck, S., Ruttens, A., Du Laing, G., Vangronsveld, J., Verloo, M.G., 2008. Chemically assisted phytoextraction: a review of potential soil amendments for increasing plant uptake of heavy metals. Int. J. Phytorem. 10 (5), 390–414. https://doi.org/10.1007/s11104-005-1755-0.

Moreno, F.N., Anderson, C.W.N., Stewart, R.B., Robinson, B.H., Nomura, R., Ghomshei, M., M., Meech, J.A., 2005. Effect of thioligands on plant-Hg accumulation and volatilisation from mercury-contaminated mine tailings. Plant Soil. 275, 233–246. https://doi.org/10.1007/s11104-005-1755-0.

Parra, R., Uley, A.L., Elles, M.P., Blaylock, M.J., 2008. Transient phytoextraction agents: establishing criteria for the use of chelants in phytoextraction of recalcitrant metals. Int. J. Phytorem. 10 (5), 415–429. https://doi.org/10.1007/s11104-008-01005-6.

Proshaskova, O., Podlahová, J., Podlaha, J., 1973. Metal complexes of thiopolycarboxylic acids. VI. Zinc and cadmium. Collect. Czech. Chem. Commun. 38, 1200–1217. https://doi.org/10.1021/cc501310x.

Qian, X., Wu, Y., Zhou, H., Xu, X., Xu, Z., Shang, L., Qiu, G., 2018. Total mercury and methylmercury accumulation in wild plants grown at wastelands composed of mine tailings: insights into potential candidates for phytoextraction. Environ. Pollut. 239, 757–767. https://doi.org/10.1016/j.envpol.2018.04.105.

Ranieri, E., Moustakas, K., Barbafieri, M., Ranieri, A.C., Herrera-Melón, J.A., Petrella, A., Tommasi, F., 2020. Phytoextraction technologies for mercury- and...
