Phytoremediation potential of aquatic plants in Uzbekistan for the treatment of cyanide-containing wastewater

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Abstract. For over a century now, the mining industry has been using cyanides for gold and silver recovery. Cyanides are highly toxic for human beings, animals, and aquatic organisms. The available physical and chemical methods of wastewater treatment are cost-ineffective. Certain microorganisms are capable of use cyanides as sources of carbon and nitrogen and turn those into ammonia and carbonate. Some plants are also efficient for the processes of cyanides destruction. Phytoremediation of cyanides may be efficient, cost-effective, environmentally friendly, and be used as an attractive alternative to traditional physical and chemical processes. This article considers the capability of aquatic plants, which grow in the valley of Zerafshan River on the territory of Uzbekistan, to dispose of cyanides and recover the cyanides-contaminated tailings of Navoi Mining & Metallurgical Combinat. Such aspects as the mechanisms of enzymatic detoxification of cyanides by aquatic plants and microorganisms are discussed. The most promising plants to be bedded in the tailings dump are selected.

Keywords: phytoremediation, cyanides, wastewater, enzymes, treatment

1. Introduction
Cyanides are highly toxic compounds widely spread in nature (cyanogenic bacteria, fungi, and plants), as well as in the zones of intensive anthropogenic load. For over a century now, starting from 1887, the mining industry has been using cyanides for gold and silver recovery. A steady trend of the growing use of cyanides is being registered around the world, including in Uzbekistan, due to the increased scope of processing the ore from primary deposits using the method of heap leaching. Every year up to 3 mln. tonnes of cyanides are used in the industry [1].

Cyanides can be found in the environment in such forms as: free acid HCN; metal salts – soluble (like NaCN and KCN) and poorly soluble and insoluble (for instance, Zn(CN)2), and metal complexes – moderately and poorly degradable. The most environmentally hazardous are “free cyanides”, and namely, cyanide ions (CN-), as well as hydrogen cyanide (HCN). Hydrogen cyanide forms a weak acid in a solution. The HCN to CN- ratio is determined by the hydrolysis reaction: \[ \text{CN}^- + \text{H}_2\text{O} = \text{HCN} + \text{OH}^- \]. The ratio of these forms strongly depends on the pH of the medium [1]. It is the free cyanides that are highly toxic for human beings, animals, and aquatic organisms [2]. They inhibit the growth and cellular metabolism, including respiration, and nitrogen and phosphates metabolism [3], they also
inhibit the mitochondrial cytochrome oxidase and cellular catalase, peroxidase, tyrosinase, ascorbic acid oxidase, and phosphatase through binding the cofactors containing metals, in metalloenzymes. CN⁻ reacts with Fe³⁺, present in the mitochondrial cytochrome oxidase and in blood, thus inhibiting oxidoreduction. As a result an organism dies. The maximum permissible concentrations of CN⁻ and their complexes equal 0.05 mg/l in Russia, and 0.1 mg/l in Uzbekistan.

Cyanides are widely spread in the vegetable kingdom and perform the defense function in the relation of plants-fungi, animals, what is one of the examples of the regulation of the interspecies interaction at the chemical level. It has been found that more than 2000 plant species are capable of cyanogenesis, that is the synthesis of glycosides. The source of hydrocyanic acid synthesis in plants are cyanohydrins, which are generated from sugars, or derivatives of amino acids, in particular of phenylalanine, or tyrosine [4-10].

The main biological function of cyanogenic glycosides is their role in defending plants against exposure to various animals (attacks from insects or herbivore). Secretion of cyanides occurs when a plant gets affected by plant pathogenic fungi, and the plant destroys the carnivore [11]. Acute poisoning of animals and human beings caused by ingestion of cyanogenic plants or food products can lead to a fast and sharp respiratory depression in mitochondria, and the consequences can be fatal. Continuous ingestion of plants with low level of cyanogenic glycosides can mostly cause specific damages of the nervous system [12-14].

The ecological role of cyanogenic glycosides is that they ensure partial, or even complete, defense against the attacks from many animals. It has been found that sheep, fallow deer, and other animals prefer feeding on non-cyanogenic plants, rodents avoid eating seeds of plants containing cyanogenic glycosides, and ants do not live in trees rich in these substances.

As a rule, the cyanogenic glycosides containing in food products yield hydrogen cyanide as a result of the process of chewing and digesting. Around 25 cyanogenic glycosides are known of, that are normally contained in edible parts of plants, such as apples, apricots, cherries, peaches, plums, and queen-apples, especially in the seeds of such fruits. Chemical substances can also be found in almonds, pits, kernels, cassava, bamboo sprouts, flax seeds, beans, chickpea, cashew, and other food plants. The maximum amounts of cyanogenic glycosides have been registered in the families of such plants as Fabaceae, Rosaceae, Leguminosae, Linaceae, and Compositae.

Cyanogenic glycoside is not toxic in and of itself. However, when a plant's cellular structures get destroyed, β-glycosidase catalyzes hydrolysis in cyanogenic glycoside. The hydrolysis results in generation of glucose and cyanohydrin, which self-decomposes into HCN and cetone or aldehyde (Figure 1).

![Figure 1. Cyanogenic glycoside.](image)

Toxicity of cyanogenic glycosides depends on the concentration of the generated cyanide and can cause acute or chronic poisoning, that is why the eating of manioc and other improperly processed products may lead to the development of tropical ataxic neuropathy (TAN), which manifests itself in optic atrophy, perceptive deafness, sensory gait ataxia, growth impairment, and development of goiter; the clinical symptoms of the cyanides toxicity include vomiting, nausea, giddiness, stomach aches, fatigue, headache, diarrhea, and sometimes, death [14].

It has been noticed that the defense by the release of HCN is not limited the vegetable kingdom. A defense pattern of centipedes holding off the attacks from ants has been described; and some of the
brightly coloured butterflies also generate HCN at all the stages of their life cycle in order to protect themselves from predators [15].

Multiple research has shown that farm livestock, as well as certain species of shellfish and snails have adapted to HCN and are capable to perform detoxification of cyanides with the help of rhodanese enzyme, which catalyzes the transformation of cyanide into thiocyanate

$$\text{CN}^- + S^+ \rightarrow N\equiv C - S^- + SO_4^{2-}$$

**Figure 2.** Detoxification of cyanide.

The sources of sulphur is $\beta$-mercaptopyroracemic acid HCH$_2$COCOOH, which in its turn transforms into pyruvate. Similar reaction is the core of the cyanide poisoning treatment, as sodium thiosulphate is injected as the antidote (1)

$$\text{CN}^- + \text{Na}_2\text{S}_2\text{O}_8 \rightarrow \text{CNS}^- + \text{Na}_2\text{SO}_3$$

Rhodanese has been thoroughly studied with regard to animals and microorganisms [16], but not that well at all in the context of plants.

There is also an option of enzymatic hydrolysis of cyanides in living organisms during the catalysis of cyanide hydratase (formamide hydrolysis) (2)

$$\text{HCN} + \text{H}_2\text{O} \rightarrow \text{HCONH}_2$$

For the first time, cyanide hydratase has been isolated from *St. loti* (plant pathogenic fungi for *Lutus corniculatu* [17]. This enzyme has also been found in some other fungi, including such strains as *F.solani*, *Gl. Sorghi*, and others. This option is typical for fungi and is the key to the microbiological treatment of wastewater off cyanides by using fungi [18-21].

Through the use of the radioisotope technique, it has been found that the generated formamide can by hydrolysis further on transform into formic acid and ammonia, which dehydrates to $\text{CO}_2$ (3).

$$\text{HCN} \rightarrow \text{HCONH}_2 \rightarrow \text{HCOOH} + \text{NH}_3 \rightarrow \text{CO}_2$$

A number of authors, through the use of $\text{H}^{14}\text{CN}$ have discovered that in higher plants, mostly legumes, as well as in some fungi the tracer carbon of cyanide is included into the carboxyl group of asparagine. The intermediate product of the assimilation of cyanide in plants is $\beta$-cyanoalanine. The enzyme that catalyzes the reaction of $\beta$-cyanoalanine synthesis from L-cysteine and cyanide is called $\beta$-cyanoalanine synthase.

$\beta$-cyanoalanine synthase ($\beta$-CAS) is a pyridoxal-dependent lyase, which catalyzes $\beta$-substitution of electrophilic groups in $\beta$-position according to this equation (Figure 3).

Scientifically discovered in 1969, $\beta$-cyanoalanine synthase is intensively studied in Russia and abroad today [22-27].

The enzyme has been isolated from plants belonging to many families, and its properties and specific features have been studied. Its other possible functions, besides the detoxification of cyanides, are being discussed.

$\beta$-cyanoalanine is quite a toxic compound, and is further transformed by plants, as catalyzed by $\beta$-cyanoalanine hydrolase ($\beta$-CAH), into an indispensable amino acid, asparagine.
The goal of this work is to perform comparative evaluation of the phytoremediation potential of various groups of aquatic plants for the purpose of selecting the most efficient plants to be bedded in the treatment facilities of gold-mining enterprises in Uzbekistan, in particular at Navoi Mining & Metallurgical Combinat.

2. Objects and methods of research

This work studies the capability of plants, which are most widely spread in the water bodies in the Republic of Uzbekistan to dispose of cyanides: we are talking about fully submerged plants or plants with their parts mostly free-floating on the surface of water, as well as floating-leaved vegetation. These are also called hydrophytes. According to the taxonomic categories, these are the representatives of various groups: bryophytes, lycophytes, and angiosperms (flowering plants).

Some of these — the most numerous group predominantly consisting of monocotyledonous plants — are fully or almost fully submerged (hydratophytes), and they include all the species which die without water and are incapable of terrestrial way of life; these plants stay in the shallows of fresh- or salt-water basins, or float on the surface.

Other species are submerged only in part of their foot end (hydrophytes), can survive a temporary drought, or require good moisturization only for their roots; those include shallow-water, coastal, and marsh species. There is no sharp boundary between hydatophytes and hydrophytes. Algae can also be roughly considered as aquatic plants.

This article studies the species of aquatic plants, which grow in the coastal strip of the middle and lower reaches of Zerafshan River: common reed (Phragmites australis (Cav.) Trin. ex Steud.), cattail (Typha latifolia L., T. angustifolia L.), flowering rush (Butomus umbellatus L.), lakeshore bulrush (Scirpus lacustris L.), spiked water-milfoil (Myriophyllum spicatum L.), coontail and soft hornwort (Ceratophyllum demersum L., C. submersum L.), pondweed (Potamogeton pectinatus L., P. crispus, P. natans L., P. perfoliatus L.), and charophytes (Chara vulgaris L., Ch. fragilis Desv., Ch. dominii Vilh.).

Special attention is drawn to charophyte algae or charophytes, as these form broad thickets on the beds of shallow and still water basins. They have a considerable impact on the dynamics of the habitat conditions of various species of hydrobionts. During the growing period these aerate water in the process of photosynthesis and sediment suspended solids on their thallomes. On the surface of algae thallomes, communities of microorganisms are usually formed, which include protozoa, microscopic periphyton algae, fungi and bacteria [28-30].

The plants have been gathered in the places of their growing, sorted out according to species, washed thoroughly, then the average sample has been taken, homogenized in buffer solution, and the
degree of activity of β-cyanoalanine synthase and β-cyanide hydratase has been evaluated, using the technique presented in work [22,23].

3. Results and discussion

The studying of the properties of β-CAS in aquatic plants has revealed that its properties fundamentally differ from the properties of this enzyme in terrestrial plants. In aquatic plants β-CAS is not related to mitochondria and is a cytoplasmatic enzyme. Such localization allows aquatic plants to quickly dispose of the exogenous cyanides coming from outside.

It has been found that β-CAS in aquatic plants depends on the pH and on the nature of buffer, and that highest activity in the reaction with KCN is registered at the pH of 8.8 in a 0.2 M pyrophosphate buffer.

To select the species of aquatic plants most efficient for the cyanides disposal, screening survey has been performed to evaluate the enzymatic activity in the aquatic plants growing in water basins in Uzbekistan.

Table 1 presents the results of evaluating the activity of two coupled enzymes participating in the detoxification of cyanides.

| Plant name                  | Activity of β-CAS, nM H₂S min⁻¹ mg⁻¹ of protein | Activity of β-CAH, nM of asparagine min⁻¹ mgr⁻¹ of protein |
|-----------------------------|-----------------------------------------------|----------------------------------------------------------|
| Fully submerged hydatophytes| 4.5                                            | 1.8                                                      |
| Nitella hyalina             | 6.8                                            | 2.8                                                      |
| Stonewort                   | 8.1                                            | 1.9                                                      |
| Brittle stonewort           | 1.2                                            | 0.9                                                      |
| Spiked water-milfoil        | 1.2                                            | 0.6                                                      |
| Coontail                    | 1.1                                            | 0.8                                                      |
| Soft hornwort               | 0.8                                            | 0.2                                                      |
| Filiform pondweed           | 0.4                                            | 0.4                                                      |
| Clasping-leaved pondweed    | 0.8                                            | 0.6                                                      |
| Fennel-leaved pondweed      | 0.5                                            | 0.4                                                      |
| White-stemmed pondweed      | 0.5                                            | 0.4                                                      |
| Floating-leaved vegetation  | 5.6                                            | 1.5                                                      |
| Flowering rush              | 2.4                                            | 1.9                                                      |
| Common reed                 | 3.2                                            | 7.0                                                      |
| Bulrush                     | 3.6                                            | 3.2                                                      |
| Narrowleaf cattail          | 2.8                                            | 1.3                                                      |
| Lakeshore bulrush           | 4.5                                            | 4.3                                                      |
| Aquarium plants             | 4.4                                            | 3.4                                                      |
| Water lettuce               | 3.2                                            | 1.0                                                      |

It has been found that the degree of activity of pyridoxal-dependent lyases depends little on the age and season, and is higher in leaves of flowering plants than in stems. In plants of the same species but growing in different ecological conditions the activity of the enzymes varies significantly. Plants growing in water basins with high insolation show higher activity, in particular, three species of pondweeds from Zerafshan River and Angara River have been compared.

The activity of β-CAH correlates with the activity of β-CAS. At high activity of β-CAS, high degree of activity of β-CAH is registered. The process of detoxification of cyanides results in generation of non-toxic metabolites of asparagine, thus the high resistance of aquatic plants to the toxicity of cyanides. It has been revealed that the concentration of sodium cyanide of 100 mg/l does not affect the growth response in plants. At the concentrations of 1-50 mg/l, on the contrary, the plants
growth intensifies and the protein content increases, as well as the level of the enzymatic activity of the enzymes under study rises. With the help of the mathematical planning of experiments, it has been experimentally found that the β-CAS induction in charophyte algae can be described by this regression equation (4).

\[ Y = 2.01 + 0.07X_1 + 0.09X_2 - 0.13X_3 - 0.05X_1^2 - 0.1X_2^2 - 0.15X_3^3 \]  

(4)

where \(X_1\) is the dose of cyanide, \(X_2\) is the \(\text{pH}\), \(X_3\) is the temperature.

Knowing the degree of activity of β-CAS, and environmental conditions in a water basin (\(\text{pH}\) and temperature), it is possible to calculate the dose of the metabolized cyanide, and therefore, the assimilation capacity of the phytoremediation treatment facilities.

4. Conclusion

In order to intensify the processes of wastewater treatment off cyanides, we may recommend to plant charophyte algae directly in the in the tailings dump, and bulrush and cattail along the banks. The assimilation capacity of the facilities should be calculated as per the proposed equation.

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Acknowledgments
The work was supported by Act 211 Government of the Russian Federation, contract No 02.A03.21.0011.