

# LIFE NARMENA - Literature Review Phytoremediation

**LIFE NARMENA -**  
**LITERATURE REVIEW**  
**PHYTOREMEDIATION**

## DOCUMENTBESCHRIJVING

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| 5  | <i>Samenvatting:</i><br>Dit document is een literatuuronderzoek waarin plantensoorten worden beschreven die geschikt kunnen zijn voor de fytoremediatie van chroom (Cr) in de Grote Calie, Turnhout, België. Naast het opsommen van chroom fytostabiliserende plantensoorten, beschrijven we aspecten van microbieel geassisteerde fytostabilisatie, microbieel geassisteerde chroomprecipitatie en de effecten van organische stof, pH en wijzigingen op chroomneerslag in de rhizosfeer. We hebben ook planten opgesomd die chroom hyperaccumuleren in de scheuten, om de lezer te informeren over welke plantensoort hij niet moet kiezen als het doel is om de verspreiding van chroom naar de omgeving te verminderen of over te brengen naar de voedselketen (landbouwgebied). Dit literatuuronderzoek werd uitgevoerd door UHasselt voor OVAM, in het kader van de LIFE NARMENA ( <u>N</u> ature-based <u>R</u> emediation of <u>M</u> etal pollutants in <u>N</u> ature <u>A</u> reas) haalbaarheidsstudie met nummer BN200108, waar bio2clean verantwoordelijk is voor de fytoremediatieprojecten. |    |   |
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## INHOUD

<b>1</b>	<b>Background, aims and objective .....</b>	<b>2</b>
1.1	Cr Contamination	2
1.2	Human Health Hazard	2
1.3	Environmental Contamination	3
1.4	Cr Chemical Properties	3
1.5	Cr Remediation Techniques	3
1.6	Cr Phytostabilization	5
1.7	Phytostabilization of wetlands	5
1.8	Microbial-assisted Phytostabilization of Cr	11
1.9	Microbial-Assisted Chromium Precipitation	12
1.10	The effects of organic matter content on Cr Precipitation	12
1.11	The effects of PH on Chromium Precipitation	13
1.12	Chromium Phytoremediation using amendments	13
1.13	Chromium Hyperaccumulators	14
1.14	Criteria for Chromium Hyperaccumulators	14
<b>2</b>	<b>References .....</b>	<b>17</b>
2.1	References Tables 1 & 2	19

## **Summary**

*The object of this document is a literature review describing plant species which may be suitable for the phytoremediation of chromium (Cr) at the Grote Calie, Turnhout, Belgium. In addition to listing Cr phytostabilizing plant species, we describe aspects of microbial assisted phytostabilisation, microbe assisted Cr-precipitation, and the effects of organic matter, pH and amendments on Cr precipitation in the rhizosphere. We also listed plants that can hyperaccumulate Cr in the shoots, in order to inform the reader which plant species not to choose if the aim is to reduce Cr distribution to the wider environment, or transfer into the food chain (agricultural area). This literature review was performed by UHasselt for OVAM, as part of the NARMENA (Nature-based Remediation of Metal pollutants in Nature Areas) feasibility study with number BN200108, where bio2clean is responsible for the phytoremediation projects.*

# 1 BACKGROUND, AIMS AND OBJECTIVE

The LIFE NARMENA project has the aim to demonstrate two nature-based remediation (NBR) methods in rivers and floodplains in nature reserves in Belgium. One of them is the phytostabilization of Cr, on the river banks of the Grote Calie, in Turnhout. The Cr input sources are no longer present but Cr remains in the sediments and on the river bank when the stream is cleaned, and polluted sediment is brought on the surface. Sometimes this sediment is unintentionally dragged further into land due to human-related activities or natural events as floods, forming a risk for uptake by agricultural crops.

Traditional remediation techniques for removing Cr are relying on the removal of the polluted matrix. These techniques are invasive, expensive and require more labour, than biological remediation approaches.

For the Grote Calie, bio2clean will use bacteria-assisted phytostabilization to lower the bioavailability and mobility of Cr and thereby decreasing the exposure and spread of this pollutant. In order to successfully apply this method, it is essential to know which plant species are most suitable for Cr retention in the roots, and which show minimal uptake of metals into the aerial parts. Prior to the field visit, we used this literature review to select plants on the field which are potentially most interesting to achieve this goal. A table is included in this review with a list of plant species (English and Dutch names) with their Cr accumulation and tolerance levels, as far as known from literature. Second, the goal of this review was to gain more knowledge on the role of bacteria, mycorrhizal fungi, but also organic matter, pH, and amendments to enhance Cr stabilisation, which will help conducting the feasibility study.

## 1.1 CR CONTAMINATION

Chromium (Cr) is an abundant heavy metal element in the Earth's mantle. It is also found in volcanic matter, soils, animals, and plants. Cr occurs naturally as chromite ( $\text{FeCr}_2\text{O}_4$ ) or complexes with other metals like lead to form crocoite ( $\text{PbCrO}_4$ ). Cr is utilized in a wide variety of industrial processes. Such processes include leather tanning, electroplating, textile production, paint and dye formulation, ceramic glazing, and inhibition of water corrosion (Oliveira, 2012, Liu *et al.*, 2020, Godlewska *et al.*, 2018).

The application of Cr in industrial processes inevitably has polluting effects on the environment by accidental and negligent mishandling Cr in industrial effluents. Anthropogenic deposition of Cr into terrestrial and aquatic ecosystems is a severe human health and ecological concern.

## 1.2 HUMAN HEALTH HAZARD

Cr can enter the food chain by its uptake in crops and waters by entering water streams. Cr is ranked seventh in the twenty most hazardous substances in the world by the Agency for Toxic Substances and Disease Registry in the USA. Additionally, Cr is classified as a human carcinogen by the International Agency for Research on Cancer (Singh *et al.*, 2013; Thangadurai *et al.*, 2020). It mainly causes lung cancer, leukemias, and gastrointestinal tumors. Chromium toxicity also causes physiological damage like skin rashes, respiratory problems like asthma or bronchitis, and nasal ulcers that affect everyday life (Linos *et al.*, 2011).

## 1.3 ENVIRONMENTAL CONTAMINATION

Cr soil pollution is an environmental and economic concern for the terrestrial ecosystem and the agricultural industry. Speciated Cr in the soil can be taken up by plants, which can be a toxic phenomenon. Plant accumulation of Cr alters its physiological activities of the plant, such as photosynthesis, water transport, and adsorption of essential micronutrients. Such interferences can lead to plant death. Additionally, in the agricultural industry, crops that take up Cr have stunted growth and poor nutritional profiles, which devalues their currency. For these reasons, mitigation of Cr contamination is a prime remediation objective (Gheju *et al.*, 2017).

Cr may enter the natural waters by being discharged from industrial operations, speciating from Cr-containing rocks, and the leaching of soils. Additionally, historical contamination of Cr and the disposal of contaminated sediments can pollute the waters and the wetlands. Wetland plants can aggravate the toxicity of trace metals by releasing exudates that alter the pH or oxidize the rhizosphere, which can affect the speciation Cr, which in turn can pollute the waters and enhance their bioavailability to other organisms. Monitoring the water stream velocity, erosion and element availability of the wetlands is another critical aspect of mitigating Cr pollution to the terrestrial and aquatic ecosystems (Santos *et al.*, 2015, Teuchies *et al.*, 2013).

## 1.4 CR CHEMICAL PROPERTIES

Chromium has two environmentally-stable isotopes: the trivalent oxidation state (Cr(III)) and the hexavalent state (Cr(VI)). The health hazards associated with chromium exposure depend on its oxidation state. Cr(VI) is the more toxic form due to its high solubility, its rapid permeability through cellular membranes, and its strong binding affinity for proteins and nucleic acids (Godlewska *et al.*, 2018). Cr(VI)'s high water solubility is the chemical property that makes it a prime concern to public health for its entry into the food chain and drinking water. The maximum allowable level of chromium in drinking water, according to the United States Environmental Protection Agency (EPA) is 100 ug/L (Singh *et al.*, 2013).

Conversely, Cr(III) is the less soluble isotope that is more likely to complex and precipitate out of solution; it adsorbs quickly on mineral surfaces or organic compounds, which makes it less bioavailable and, therefore, less toxic. Cr(III) is a beneficial micronutrient to humans and animals by enhancing insulin sensitivity in diabetics and by assisting in the metabolism of macronutrients (Godlewska *et al.*, 2018). Unlike Cr(VI), Cr(III) is passively diffused through cellular membranes and forms stable complexes with nucleic acids and proteins; it is less permeable to cell walls than Cr(VI). Cr(III) mobility decreases under highly acidic conditions and with clay adsorption.

## 1.5 CR REMEDIATION TECHNIQUES

### 1.5.1 Chemical remediation

Conventional remediation of Cr(VI) from industrial effluents involves its chemical immobilization and reduction into the less toxic Cr(III). Chemical treatment by sulphur or iron-based compounds like calcium polysulphide and amorphous iron sulphide are example additives applied to contaminated sites for cleaning. Chemical reduction, although practical, can be pricey and cause downstream environmental damage on-site (Dhal *et al.*, 2013).

## 1.5.2 Phytoremediation

Green remediation technology, like phytoremediation, is increasingly applied to remove heavy metal contamination from terrestrial and aquatic ecosystems. Phytoremediation is the practice of utilizing plants and their associated microorganisms to reduce and detoxify contaminants like Cr in the environment (Gheju *et al.*, 2017).

Phytoremediation has the following advantages over conventional chemical techniques:

- 1 Cost-effective restoration technology.
- 2 Monitoring and maintenance of the site demand fewer labour hours.
- 3 An environmentally sustainable technique, less invasive than traditional civil engineer-based ones.
- 4 It can be integrated into other vegetation and landform design strategies and programs.

Limiting factors of phytoremediation technology include a slower reclamation time due for instance to the growth rate of plants and their growing season, a shorter depth of reclamation below ground, and partial reclamation due to limited bioavailability of the pollutant. For a phytoremediation approach to be successful the pollutants must be available to plants. This means that depending on how deep the pollution is located in the soil and/or (ground)water the most appropriate vegetation mix should be chosen. For instance, poplars have a root system which reaches up to 10 meters and are suitable plants for detoxifying groundwater flows. Depending on the remediation objective and chosen phytotechnology mechanism, the remediation duration will vary. Phytostabilization focuses on the establishment of a plant cover to immobilise metals in the soil or on talluds, with the aim of reducing the risk of further spread of the pollutants, and restoring the environmental status of a polluted soil to make it useable for private or public applications. Compared to phytoextraction, phytostabilization is much faster, and can already lead to some beneficial effects within one to few years.

Chromium is often phytoremediated by:

- 1 Phytostabilization:
  - a. *in situ* action, where the contaminant is detoxified and prevented from being translocated to the aerial parts of the plants;
  - b. The NARMENA project will apply phytostabilization to restore the Cr-contaminated site (see the section, Chromium Phytostabilization details).
- 2 Phytoextraction:
  - a. The process of removing heavy metal contaminants using (hyper)accumulator plants that can take up a large amount of the metal into their shoot system;
  - b. Phytoextraction is especially a sustainable technology for low-medium contaminated sites.



## 1.6 CR PHYTOSTABILIZATION

Remediation is approached with phytostabilization when large-scale areas of soil are contaminated with high concentrations of trace elements. Under high Cr(VI) conditions, plant growth is inhibited and therefore, phytoextraction is not a sustainable solution. Phytostabilization is a management strategy, which focuses on the long-term stabilization and containment of a trace element pollutant by using vegetation. Additionally, it prevents toxic metal forms from entering the food chain, by crop accumulation into the shoots, and our drinking waters. Phytostabilization utilizes plants and their associated microbes to retain contaminants, prevent their further dispersal, and decrease their bioavailability to the plant for uptake (Bolan *et al.*, 2011, Singh *et al.*, 2013).

The stabilization of Cr can be achieved by:

- 1 Detoxifying Cr(VI) into Cr(III) (see the section, Microbial-assisted reduction of Cr(VI)).
- 2 Immobilizing Cr(III) by precipitating out of solution and forming stable, solid complexes (see the section, Microbial-assisted Cr precipitation).
- 3 Using biosorbents to bioaccumulate Cr onto their surface.

## 1.7 PHYTOSTABILIZATION OF WETLANDS

Contaminated wetlands are often remediated with phytostabilization. In most wetlands, heavy metals are more bioavailable, where they frequently move from the sediments to the aboveground plant tissues. Their easy translocation to plant shoot systems can be toxic to the plants. The physicochemical traits of the belowground system affect metal mobility and hence their bioavailability.

Wetland plants are rooted in anoxic sediments, but they often maintain an aerobic root respiration system. These plants affect the redox potential in the rhizosphere as roots release oxygen into the system. When plant roots accumulate an excess of oxygen, they leak it out and consequently oxidize the rhizosphere. Oxidation alters the soil pH, which in turn affects metal mobility and bioavailability (see the section, The Effects of pH on Chromium Precipitation). Cr movement in contaminated sediments is similar to high zinc and cadmium contaminations in a marsh that infected willow trees (*Salix* sp.) (Teuchies *et al.*, 2013, Vandercasteele *et al.*, 2002). If they are not immobilized effectively, the metals will continue to move from the sediments, and transfer up to the shoot system.

Additionally, biosorption of Cr involves an ion-exchange mechanism with a biosorbent and has applications in wetland settings. For example, wetland plants like green macroalga, *Cladophora glomerata*, and marine macroalgae *Enteromorpha prolifera* accumulate Cr(III) ions onto the surface of their leaves (Godlewska *et al.*, 2018). Biosorption is practical under conditions where Cr(III) cannot precipitate out of solution (see section, The Effects of pH on Chromium Precipitation). The biosorption of Cr(III) is generally very rapid in the beginning when the concentration of Cr(III) is high in solution, and it gradually decreases as the plant reaches a homeostatic state with the ions (see table 1).

Plant Species	Dutch name	Plant family	Soil type – substrate	Cr in soil (mg/kg)	Cr in root mg/kg DW	Cr in shoot (mg/kg DW)	TF	Mechanism and extra info	Reference
<i>Salix matsudana</i>	Wilg	Salicaceae (wilgenfamilie)	N/A	NA	746	NA			Yu <i>et al.</i> , 2008
<i>Salix babylonica</i>	Wilg	Salicaceae (wilgenfamilie)	N/A	NA	559	NA		Root uptake	Yu <i>et al.</i> , 2008
<i>Ailanthus altissima</i>	Hemelboom	Simaroubaceae (hemelboomfamilie)	N/A	NA	358	NA		Root uptake	Ranieri <i>et al.</i> , 2016
<i>Cichorium spinosum</i>	Cikorie	Asteraceae (composietenfamilie)	Manure	NA	300	NA		Precipitation; organometallic complexes	Antoniadis <i>et al.</i> , 2017
<i>Pluchea indica</i>	Indiase kamferwier	Asteraceae (composietenfamilie)	N/A	NA	152	NA		Root uptake, but also translocation to shoot	Sampanpanish <i>et al.</i> , 2006
<i>Cynodon dactylon</i>	Handjesgras	Poaceae (grassenfamilie)	N/A	NA	152	NA		Root uptake, translocation into shoots	Shahandeh <i>et al.</i> , 2000
<i>Helianthus annuus</i>	Gewone zonnebloem	Asteraceae (composietenfamilie)	Sandy soil	NA	49.1	NA	0.49	Root uptake, 64.8 % removal by root, Arbuscular Mycorrhizal fungi (AMF) could enhance Cr tolerance of sunflower	Dong <i>et al.</i> , 2007
<i>Panicum antidotal</i>	Vingergras	Poaceae (grassenfamilie)	Clayey soil	NA	43.3	NA	0.1	Root uptake, 68.5 % removal by root	Shahandeh <i>et al.</i> , 2000
<i>Pennisetum purpureum</i>	Olifantsgras	Poaceae (grassenfamilie)	Sandy soil	NA	33.8	NA	0.48	Root uptake, 66.8 % removal by root	Juel <i>et al.</i> , 2018
<i>Gossypium hirsutum</i>	Behaarde katoen	Malvaceae (kaasjeskruidfamilie)	Sandy soil	NA	31.4	NA	0.52	Root uptake, 65.1 % removal by root	Lofty <i>et al.</i> , 2014
<i>Cucurbita pepo</i>	Sierpompoe	Cucurbitaceae	Clayey soil	NA	23.5	NA	0.54	Root uptake, 65 % removal by root	Lofty <i>et al.</i> , 2014

Plant Species	Dutch name	Plant family	Soil type – substrate	Cr in soil (mg/kg)	Cr in root mg/kg DW	Cr in shoot (mg/kg DW)	TF	Mechanism and extra info	Reference
<i>Alisma sp.</i>	Weegbreesoort	Alismataceae	Tidal Marsh of Schelde estuary	130	NA	5.18			Teuchies <i>et al.</i> , 2013
<i>Pulicaria dysenterica</i>	Heelblaadjes	Asteraceae (composietenfamilie)	Tidal Marsh of Schelde estuary	130	NA	5.43			Teuchies <i>et al.</i> , 2013
<i>Bidens cernua</i>	Knikkend Tandzaad	Asteraceae (composietenfamilie)	Tidal Marsh of Schelde estuary	130	NA	1.36			Teuchies <i>et al.</i> , 2013
<i>Cirsium arvense</i>	Kruipende distel	Asteraceae (composietenfamilie)	Tidal Marsh of Schelde estuary	130	NA	7.17			Teuchies <i>et al.</i> , 2013
<i>Bidens frondosa</i>	Zwart tandzaad	Asteraceae (composietenfamilie)	Tidal Marsh of Schelde estuary	130	NA	3.42			Teuchies <i>et al.</i> , 2013
<i>Symphytum officinale</i>	Gewone smeerwortel	Boraginaceae (ruwbladigenfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.82			Teuchies <i>et al.</i> , 2013
<i>Scirpus maritimus</i>	Zeebies	Cyperaceae (zeggefamilie)	Tidal Marsh of Schelde estuary	130	NA	2.06			Teuchies <i>et al.</i> , 2013
<i>Juncus effusus</i>	Pitrus (gewone of zachte rus)	Juncaceae (russenfamilie)	Tidal Marsh of Schelde estuary	10	NA	1.93			Teuchies <i>et al.</i> , 2013
<i>Juncus maritimus</i>	Zeerus	Juncaceae (russenfamilie)	Tidal Marsh of Schelde estuary	130	NA	0.75			Teuchies <i>et al.</i> , 2013

Plant Species	Dutch name	Plant family	Soil type – substrate	Cr in soil (mg/kg)	Cr in root mg/kg DW	Cr in shoot (mg/kg DW)	TF	Mechanism and extra info	Reference
<i>Juncus articulatus</i>	Zomprus	Juncaceae (russefamilie)	Tidal Marsh of Schelde estuary	130	NA	4.66			Teuchies <i>et al.</i> , 2013
<i>Lycopus europaeus</i>	Wolfspoot of zigeunerkruid	Lamiaceae	Tidal Marsh of Schelde estuary	130	NA	2.42			Teuchies <i>et al.</i> , 2013
<i>Lythrum salicaria</i>	Grote kattenstaart	Lythraceae (kattenstaartfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.2			Teuchies <i>et al.</i> , 2013
<i>Epilobium hirsutum</i>	Harig wilgenroosje	Onagraceae	Tidal Marsh of Schelde estuary	130	NA	1.65			Teuchies <i>et al.</i> , 2013
<i>Veronica beccabunga</i>	Beekpunge	Plantaginaceae (weegbreefamilie)	Tidal Marsh of Schelde estuary	130	NA	7.33			Teuchies <i>et al.</i> , 2013
<i>Veronica anagallis-aquatica</i>	Blauwe waterereprijs	Plantaginaceae (weegbreefamilie)	Tidal Marsh of Schelde estuary	130	NA	8.44			Teuchies <i>et al.</i> , 2013
<i>Plantago major</i>	Grote weegbree	Plantaginaceae (weegbreefamilie)	Tidal Marsh of Schelde estuary	130	NA	8.03			Teuchies <i>et al.</i> , 2013
<i>Poa sp.</i>	Beemdgrassoort	Poaceae (grassenfamilie)	Tidal Marsh of Schelde estuary	130	NA	4.61			Teuchies <i>et al.</i> , 2013
<i>Phragmites australis</i>	Gewoon riet	Poaceae (grassenfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.98			Teuchies <i>et al.</i> , 2013

Plant Species	Dutch name	Plant family	Soil type – substrate	Cr in soil (mg/kg)	Cr in root mg/kg DW	Cr in shoot (mg/kg DW)	TF	Mechanism and extra info	Reference
<i>Glyceria maxima</i>	Liesgras	Poaceae (grassenfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.98			Teuchies <i>et al.</i> , 2013
<i>Phalaris arundinacea</i>	Rietgras of kanariegras	Poaceae (grassenfamilie)	Tidal Marsh of Schelde estuary	130	NA	5.28			Teuchies <i>et al.</i> , 2013
<i>Polygonum lapathifolium</i>	Beklierde duizendknoop	Polygonaceae (duizendknoopfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.94			Teuchies <i>et al.</i> , 2013
<i>Rumex conglomeratus</i>	Krulzuring	Polygonaceae (duizendknoopfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.47			Teuchies <i>et al.</i> , 2013
<i>Rumex obtusifolius</i>	Ridderzuring	Polygonaceae (duizendknoopfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.7			Teuchies <i>et al.</i> , 2013
<i>Persicaria hydropiper</i>	Waterpeper	Polygonaceae (duizendknoopfamilie)	Tidal Marsh of Schelde estuary	130	NA	2.33			Teuchies <i>et al.</i> , 2013
<i>Ranunculus repens</i>	Kruipende boterbloem	Ranunculaceae	Tidal Marsh of Schelde estuary	130	NA	7.76			Teuchies <i>et al.</i> , 2013
<i>Salix sp.</i>	Wilg	Salicaceae	Tidal Marsh of Schelde estuary	130	NA	1.4		S.viminalis had high affinity in chromium retaining in all plant tissues	Teuchies <i>et al.</i> , 2013, Ranieri <i>et al.</i> , 2014

Plant Species	Dutch name	Plant family	Soil type – substrate	Cr in soil (mg/kg)	Cr in root mg/kg DW	Cr in shoot (mg/kg DW)	TF	Mechanism and extra info	Reference
<i>Typha latifolia</i>	Grote lisdodde	Typhaceae (lisdoddefamilie)	Tidal Marsh of Schelde estuary	130	NA	1.34			Teuchies <i>et al.</i> , 2013
<i>Urtica dioica</i>	Gewone netel	Urticaceae (netelfamilie)	Tidal Marsh of Schelde estuary	130	NA	4.63			Teuchies <i>et al.</i> , 2013

Table 1: Overview of Cr tolerant and potential phytostabilizer plants. Individuals of the green marked plants were sampled at the nature reserve area or agricultural site, at the Grote Calie, Turnhout on June 19, 2020.

### 1.7.1 Phytostabilizers

A robust phytostabilizer should particularly have high root biomass with the ability to immobilize the contaminant or to hold them in the roots. The efficacy of phytostabilization can be measured by:

- 1 Mobility of metals in the rhizosphere
  - a. Measured by the bioconcentration factor (BCF), which is the ratio of metal concentration in the plant's shoots to the metal concentration in the soil.
  - b.  $BCF < 1$  then the plant is more suitable for phytostabilization
- 2 Translocation efficiency from the roots to shoots
  - a. The ratio of metal concentration in the shoots to that of the roots.
    - $TF < 1$ , then the plan is suitable for phytostabilization (Singh *et al.*, 2013).

## 1.8 MICROBIAL-ASSISTED PHYTOSTABILIZATION OF CR

Immobilization of Cr is one method of mitigating its contamination in the natural environment. Soil bacteria play a significant role in this immobilization process by first detoxifying Cr(VI) and then making it less soluble through bacterial-assisted reduction of Cr(VI) to Cr(III) and precipitation of Cr(III), respectively. Soil bacteria benefit from living associated with plants because of higher nutrient availability and plants require plant growth promotion bacteria for the same reasons (Focardi *et al.*, 2013; Lin *et al.*, 2019). In conclusion, microorganisms remediating chromium contamination are not mutually exclusive from plants but rather work with them in an integrated and synergistic system.

### 1.8.1 Microbial-assisted reduction of Cr (VI)

Biotransformation of Cr(VI) to Cr(III) by bacteria is a process that involves an enzymatic reduction of Cr(VI), utilizing Cr(VI) as a final electron acceptor. Chromate reductase is an enzyme that is produced by Cr-resistant bacteria and can be exploited for remediation purposes (Mishra *et al.*, 2012).

Numerous bacteria can reduce Cr(VI) to Cr(III) with reductase catalytic activity:

- 1 *Pseudomonas putida*
- 2 *Escherichia coli*
- 3 *Desulfovibrio sp.*
- 4 *Bacillus sp.*
- 5 *Shewanella sp.*
- 6 *Arthrobacter sp.*
- 7 *Streptomyces sp.*
- 8 *Microbacterium sp.*
- 9 *Staphylococcus aureus*
- 10 *Pediococcus Pentosaceus*

*S. aureus* and *P. pentosaceus* are strains previously found in tannery effluents collected from drain waters. Their chromate reductase activity can reduce more than 95% of Cr(VI) in the toxic concentration range (Bolan *et al.*, 2011; Mishra *et al.*, 2012 ). Some of these remediating bacteria, like the *Pseudomonas* and *Bacillus* sp., are reported as plant growth-promoting the release of phytohormones that help plants grow, enabling roots to exude more nutrients beneficial to these bacteria. *Bacillus* sp. alone reduces up to 75% under appropriate conditions (see the section, The Effects of pH on chromium precipitation) (Upadhyay *et al.*, 2017).

Cr(VI) can be toxic to the soil microbial diversity and impede on the nutritional status of the community. Chromium resistant bacteria can overcome these toxic effects and assist in remediation simultaneously (Smith *et al.*, 2000). Reducing Cr(VI) to Cr(III) is a favorable process in that Cr(III) is less soluble and more likely to complex out of solution through a precipitation process.

Alternatively, Cr-tolerant fungi can either reduce Cr(VI) into its trivalent form or take up Cr directly, removing it from the environment. Some like *Fusarium chlamydosporium* have been isolated from tannery wastewaters. Other examples of fungi with remedial capabilities of Cr are *Aspergillus* sp., *Rhizopus* sp, *Humicola grisea*, and *Nannizzia* sp. These fungi can remediate heavily contaminated sites where Cr(VI) concentrations go as high as 300mg/L and 400 mg/L. Generally, filamentous fungi are found to exhibit Cr-tolerance, especially those already found in polluted sites (Smith *et al.*, 2000). Wu *et al.*, 2016 described that chromium immobilization by extraradical mycelium of arbuscular mycorrhiza contributes to plant Cr tolerance.

## 1.9 MICROBIAL-ASSISTED CHROMIUM PRECIPITATION

Cr precipitation is crucial to remediation in that it first decreases its availability to plants, which detoxifies the site, and secondly, under proper conditions, it sustains Cr in a solid phase to prevent plant uptake over time. Relative to Cr(VI), Cr(III) will easily precipitate out of solution and remain in a complex solid with stability. Bacteria can promote precipitation of Cr(III) by:

- 1 Producing acids, like sulfuric acid by *Thiobacillus* sp., to create a favourable acidic environment (see section The Effects of pH on chromium precipitation, for details).
- 2 Producing chelating agents that bind Cr(III) to organic compounds.
- 3 Producing ammonia that precipitates Cr(III) into a gray-green hydroxide.

To maintain Cr as a precipitate, appropriate environmental conditions must be set in place, monitored and maintained over time.

## 1.10 THE EFFECTS OF ORGANIC MATTER CONTENT ON CR PRECIPITATION

Cr bioavailability depends heavily on the soil's organic matter content. Chromium has a high affinity for organic matter. Under low organic matter conditions, sediments cannot effectively immobilize the metal, and, as a result, chromium's bioavailability increases. Conversely, higher organic matter contents have higher microbial activity, which leads to higher sulphide concentration and a more reduced state of sediment. Under these conditions, Cr precipitates as sulphide, which reduces its bioavailability to the plant.



A study by Du Laing *et al.*, 2009 studied 26 wetland plants in the sediment along the Scheldt river, where they found the average concentration of chromium in sediment to be 134 mg/kg DM. The average Cr content in the roots of reed plants (*Phragmites australis*) was 400 µg/kg DM (see also Table 1).

Cr bioavailability can increase due to:

- 1 Degradation of organic matter.
- 2 Oxidation of metal sulphides.
- 3 Solubilization of manganese and iron hydroxides.

In conclusion, Cr stabilization mainly relies on a high organic matter concentration, reduction of metal sulphides, and removing essential compounds (Amato *et al.*, 2016).

## 1.11 THE EFFECTS OF PH ON CHROMIUM PRECIPITATION

The literature of Cr remediation suggests that precipitation is optimal under low acidic to neutral pH conditions. Additionally, it highlights the effects of microbial communities on establishing and modifying the pH by releasing exudates that can either promote a remedial setting for Cr precipitation or impede it. For instance, precipitation studies showed how a rhizobacteria consortium of sunflower (*Helianthus annuus*), can effectively remove Cr(VI) out of solution when pH is above 4.

Conversely, Cr(VI) is mobilized in both highly acidic and alkaline soil pH. Cr cannot precipitate under highly acidic conditions (pH < 4). Instead, it forms into chromic acid (HCrO<sub>4</sub>). Therefore, it requires a bacterial-assisted pH increase (Chen *et al.*, 2003). Additionally, pH influences site dissociation where low acidity (pH 5) decreases Cr(VI) biosorption capacity and hence, the concentration of metal in speciation. At pH 5.5, Cr(III) can precipitate as chromium oxide. Under conditions where the pH is 5, biosorbents of Cr(III) are thus applied to tackle the ions that will not precipitate.

Ilias *et al.* (2011) paper on Cr(VI) reduction found that *P. pentosaceus* and *S. aureus* growth and chromate reductase activity was optimal between a pH of 7.0 and 8.0. Above pH 8.0, the reduction activity significantly declined. Other strains like *Bacillus* and *P. synxantha* isolates operated optimally at similar pH ranges (Ilias *et al.*, 2011). Potting experiments using barley showed how plant yields were affected by soil pH in pots contaminated with Cr(VI) whereas in soils contaminated with Cr(III) yields were influenced by the soil pH and Cr dosage.

## 1.12 CHROMIUM PHYTOREMEDIATION USING AMENDMENTS

Phytoremediation is a slow remediation process. For this reason, soil amendments are applied to enhance the rate of phytostabilization and achieve desired results faster. Commonly used soil amendments are biocompost, zeolite, phosphate fertilizers, sawdust, and limestone. Amendments can assist Cr(VI) remediation by:

- 1 Reduction to Cr(III) by increasing organic matter content.
- 2 Retention by applying high-binding capacity materials to the soil.

Amendments like zeolites are shown to effectively induce Cr reduction and retain Cr(III) in the zeolite pores. Additionally, the application of organic amendments, like manure, shows strong Cr remedial effects, low levels of Cr uptake, by forming Cr-organic complexes that are too high in molecular weight to be taken up by any root (Antoniadis *et al.*, 2017).

## 1.13 CHROMIUM HYPERACCUMULATORS

Metallophytes, commonly known as hyperaccumulators, are plants that grow on metalliferous soils and that can uptake and cope with copious amounts of metals in their tissues. They are an inexpensive solution to remediating contaminated sites by trace elements. Their only disadvantage is their commonly slow growth and their small size, which limits their speed of metal removal from the ground (Prasad *et al.*, 2005). Conversely, non-metallophytes are faster growing, higher biomass with an in-depth root system. These plant types can accumulate a wide range of heavy metals and are an excellent supplement to hyperaccumulators. Often, they are utilized in contaminated soils, sediments, and waters where chromium isn't the only metal to be removed (Ranieri *et al.*, 2014).

Chromium hyperaccumulators use a chemical process called selective chelation, where they mobilize the metal from the soil by secreting chelating agents that have a high affinity for chromium and facilitate their uptake by roots. Once in the roots, Cr(VI) is often sequestered in vacuoles into its less toxic form, Cr(III). Translocation of Cr from the root to the shoot system (Oliveira 2012; Shahid *et al.*, 2017).

## 1.14 CRITERIA FOR CHROMIUM HYPERACCUMULATORS

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Plant Species	Common Plant Name	Plant Cr tolerance (mg per kg of tissue)	Uptake Location	Type of accumulator
<b>Cr (VI)</b>				
<i>Leptospermum scoparium</i>	Tea Tree	20,000	Foliage ash	Very high
<i>Azolla</i> spp. ( <i>A. filiculoides</i> , <i>A. microphylla</i> , <i>A. pinnata</i> )(min)	Fern	5000	Whole plant	High
<i>Azolla</i> spp. ( <i>A. filiculoides</i> , <i>A. microphylla</i> , <i>A. pinnata</i> ) (max)		15,000	Whole plant	High
<i>Eichhornia crassipes</i> (max)	Water Hyacinth	6,000	Roots	High
<i>Eichhornia crassipes</i> (min)	Water Hyacinth	3,900	Roots	Moderate
<i>Azolla caroliniana</i>	Fern	356	Whole plant	Low
<i>Brassica juncea</i>	Rapeseed	5740	Whole plant	High
<i>Brassica napus</i>	Rapeseed	306.1	Whole plant	Low
<i>Callitriche cophocarpa</i>	Water Starwort	1000	Shoots	Low
<i>Convolvulus arvensis</i>	Bindweed	3000	Leaves	Moderate
<i>Gynura pseudochina</i>	Succulent	1611	Whole plant	Low
<i>Helianthus annuus</i>	Common Sunflower	1912	Whole plant	Low
<i>Leersia hexandra</i>	Swamp Rice Grass	8938	Whole plant	High
<i>Lemna minor</i>	Duckweed	2870	Plant Tissue	Moderate
<i>Marsilea drummondii</i>	Fern	1300	Roots	Low
<i>Myriophyllum brasiliense</i>	Brazilian Watermilfoil	1770	Roots	Low
<i>Najas indica</i>	Guppy grass	458	Leaves	Low
<i>Phragmites Australis</i>	Common Reed	1919	Roots	Low
<i>Polygonum hydropiperoides</i>	Swamp Smartweed	2980	Roots	Moderate
<i>Prosopis juliflora</i>	Shrub	372	Whole plant	Low
<i>Prosopis laevigata</i>	Flowering Tree	13551	Whole plant	High
<i>Pteris vittata</i>	Chinese Brake	6862	Whole plant	High
<i>Salsola kali</i>	Saltwort (S. Tragus - Ontario Wildflower)	4290	Whole plant	High
<i>Salvinia natans</i>	Floating Fern/Watermoss	12600	Whole plant	High
<i>Spartina argentinensis</i>	Tall Grassland	15100	Whole plant	Very high
<i>Thlaspi caerulescens</i>	Alpine Pennygrass	3400	Whole plant	Moderate
<b>Cr (III)</b>				
<i>Genipa americana</i>	Tropical Tree	3841	Root	Moderate
<i>Salix babylonica</i>	Chinese Willow Tree	2111	Roots	Moderate
<i>S. matsudana</i>	Chinese Willow Tree	2624	Roots	Moderate
<i>S. matsudana</i> x <i>S. alba</i>		1235	Roots	Low
<b>Total Cr [Cr (III) + Rr (VI)]</b>				
<b>Tannery sludge* ( <i>Avena sativa</i>, <i>Triticum aestivum</i>, <i>Sorghum bicolor</i>, <i>Sorghum sudanese</i>)</b>	Common Oat Bread Wheat Grass Species Often Used In Ontario	10150	Shoots	High

Table 2: List of chromium hyperaccumulators, their capacity of chromium uptake, and their localization in the plant (Singh et al. 2013).

Reference Table	
[Cr] Ranges	Type of Accumulator
> = 15000	Very High
> = 4000, < 15000	High
< 4000, > 2000	Moderate
< 2000	Low

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