The Induced Accumulation Of Gold In The Plants *Brassica juncea, Berkheya coddii* And Chicory

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Abstract:
In this study the growth substrate of the plants Brassica juncea, Berkheya coddii and chicory were amended with thiocyanate and cyanide solutions to induce uptake and the gold concentrations in the different organs determined. Both species showed maximum uptake with cyanide amendment although thiocyanate also induced hyperaccumulation. Gold concentrations ranged from negligible in the leaves of B. coddii amended with thiocyanate, to 326 mg Au/kg dried biomass in the leaves of B. juncea amended with cyanide. The chemical additives KI, KBr, NaS_2O_3, were also used with the B. juncea and chicory. The results showed varying degrees of hyperaccumulation with all chemical treatments. Cyanide again gave the best results with 164 mg Au/kg dried biomass measured in the chicory plant. NaS_2O_3, KI and NaSCN gave maximum results of 51, 41, and 31 mg Au/kg dried biomass respectively. This technology has potential application in the economic recovery of metals.
Keywords: hyperaccumulation, gold, phytoremediation.

Introduction

The field of metal accumulation and hyperaccumulation by plants is a young one and the specific area of gold hyperaccumulation barely nascent. Currently, the main interest in metal accumulators lies in the field of phytoremediation where plants are used to 'clean up' metal contaminants from the soil.

However, for some metals it is possible that hyperaccumulation could be an economic means of mining the metal (phytomining), either because they are accumulated in very large amounts in certain plants or because they are highly valuable.

The term hyperaccumulation has come to represent a value 1000 times the highest found in non-accumulating plants. For gold this defines a threshold at 1 mg/kg [1]. By 1997 plants had been discovered that will hyperaccumulate Cd, Co, Cu, Mn, Ni, Se, Tl, and Zn [2]. The number of Ni hyperaccumulating plants stands at 317, by far the largest number found for a given metal.

There have been no discoveries to date of plants that will naturally hyperaccumulate gold. This can be accredited to the fact that gold exists as an insoluble species in soil solution and is, therefore, unavailable for plant uptake.

Shacklette *et al.* [3] were the first to investigate the hydroponic uptake of gold by plants, in particular in *Impatiens holstii* and *Impatiens balsamina*. Gold cyanide, bromide, iodide, thiocyanate, and thiosulfate solutions were trialled and all of the plants exhibited accumulation of gold to some extent. Plants could therefore accumulate gold if a soluble species were available. The next step was to solubilise metals in the soil solution.

This idea of solubilising an intractable species by introducing a chemical agent to the soil found its main application in the area of phytoremediation of such insoluble pollutants as lead [4, 5, 6]. Several articles have since been published which detail experiments showing that plants can be induced to accumulate or even hyperaccumulate gold with the addition of sodium thiocyanate solution to the substrate [1, 7, 8, 9].

The objective of this study was to further investigate the effect of chemical addition to gold bearing soils on the uptake of gold by plants. The effect of thiocyanate on uptake by *Brassica juncea*, *Berkheya coddii* and chicory was determined. Also the effect of iodide, bromide, cyanide, thiocyanate and thiosulfate was investigated for *B. juncea* and chicory.

Materials and Methods

Soil and Chemical Preparation

Artificial gold-bearing soil was prepared by dripping a 1000 µg/mL gold chloride solution onto silica sand sieved to <200 µm. The sand was then dried and diluted with fresh river sand to give a 5 µg/g (5 ppm) substrate.

The chemical solutions used were prepared from laboratory grade reagents by dissolving the appropriate amount in tap water. The chemicals used were NaSCN, KCN, KI, KBr, $(NH_4)_2S_2O_2$.

Plant Trials

In each experiment, seedlings of the plant used were transferred to pots containing artificial substrate and allowed to grow for 4-6 weeks. At this point the soils were amended with controlled amounts of the solubilising agents and the plants left for another week, upon which time they were harvested.

B. Juncea *with NaSCN and KCN*

Nine *B. juncea* plants were grown. Their soil was amended with 0.5 g/kg substrate and 1.0 g/kg substrate thiocyanate,

with four replicates in each set. One was treated with 1.0 g/kg potassium cyanide. Each plant was separated into leaves, stems and roots for separate analysis.

B.Coddii *with NaSCN or KCN*

Five *B. coddii* specimens were grown. The soil was amended with 0.5 g/kg substrate and 1.0 g/kg substrate sodium thiocyanate with two replicates in each set and one with 1.0 g/kg substrate cyanide. Each plant was separated into leaves, stems and roots for separate analysis.

B. Juncea *and Chicory with* $(NH_4)_2S_2O_2$ *KBr, KI, KCN, or NaSCN*

This treatment was carried out on both *B. juncea* and chicory. The plants were treated with bromide, iodide, cyanide, thiocyanate, and thiosulfate, with four replicates in each set for *B. juncea*. There was one chicory plant in each set excepting thiocyanate for which there were four. For both species there was one control.

Analysis

Each sample was dried, ground, then a portion ashed at 550 °C for analysis. The ashed sample was then dissolved in hot 2 M HCl. This solution was then extracted quantitatively into methyl isobutyl ketone (MIBK) by shaking for 5 minutes. After dilution with MIBK (if necessary) the organic layer was analysed using *G*raphite *F*urnace *A*tomic *A*bsorption *S*pectroscopy (GFAAS) and the concentration of the gold in the original sample determined.

Results and Discussion

B. Juncea *with NaSCN and KCN*

The concentration of gold in the thiocyanate treated plants increases from leaves to stems to roots (Table 1). It can also be seen that for the plant treated with cyanide the reverse is true, with the highest concentration in the leaves. In all cases the concentrations in the plant overall are well above the 1.0 mg/kg threshold for hyperaccumulation.

Table 1: *Average gold concentration in B. juncea amended with thiocyanate (for 1.0 and 0.5 g* SCN^-*/kg dried biomass)*

	Gold uptake by plant (mg Au/kg dried biomass)		
Plant Organ	CN^-	SCN^-	
	1.0 g/kg	1.0 g/kg	0.5 g/kg
Leaves	326	15	4
Stems	46	62	9
Roots	88	172	36

The plants amended with 0.5 g/kg substrate thiocyanate had a much lower gold concentration in all organs but hyperaccumulation still occurred.

B. Coddii *with NaSCN or KCN*

The concentration of gold in *B. coddii* was found to be highest in the roots of the SCN^- amended plants and in the leaves of the CN^- amended plant. And again a higher SCN^- loading gives better uptake, although in both cases only the roots showed significant accumulations of gold (Table 2).

Table 2: *Gold uptake by B.coddii treated with thiocyanate (for 1.0 and 0.5 g SCN/kg dried biomass).*

	Gold uptake by plant (mg Au/kg dried biomass)		
Plant Organ	CN^-	SCN^-	
	1.0 g/kg	1.0 g/kg	0.5g/kg
Leaves	97	0.31	<0.01
Stems	94	<0.01	<0.01
Roots	36	49	31

B. Juncea *and Chicory with KBr, KI, KCN, or NaSCN*

B. juncea showed increasing hyperaccumulation in the order KBr < NaSCN < KI ≈ $(NH_4)S_2O_3$ < KCN, and it can be seen that chicory followed much the same trend (Table 3). In this third series of experiments the leaves of *B. juncea* did not exhibit a higher concentration than in the roots for the cyanide-amended plant as was seen in the other trial. This is surprising as in other recent work not reported here we have found with cyanide the leaves tend to have the highest concentrations of gold.

Table 3: *Average gold uptake with varying chemical treatments*

Chemical Additive (0.5 mg/kg growth substrate)	Gold uptake by plant (mg Au/kg dried biomass)		
	B. juncea		Chicory
	Leaves	Roots	Whole Plant
KBr	5	7	17
NaSCN	4	24	31
KI	14	38	41
$(NH_4)_2S_2O_3$	15	22	52
KCN	16	76	164
Control	-	3	7

The high values for the controls are likely a combination of some contamination and the analytical uncertainty.

DISCUSSION

The plants grown in growth substrate amended with solubilisers, other than cyanide, all showed the same trend of the highest gold concentration in the roots, with lower concentrations in the stems and leaves.

A comparison between trials gives an idea of the reproducibility of the results. The gold uptake for a thiocyanate loading of 0.5 g/kg substrate were 4 and 36 mg Au/kg dried biomass for the leaves and roots respectively for the first trial (Table 1). For the same thiocyanate loading in the second trial loadings of 24 mg Au/kg dried biomass for the leaves and roots were obtained (Table 2). Given the variability of the growing conditions that the plants experienced, these are very close results. More work needs to be done on establishing standard conditions for plant growth.

Thiocyanate amendment resulted in hyperaccumulation in both *B. juncea* and *B. coddii*. Substrate amendment at a rate of 1.0 g NaSCN/kg growth substrate gave higher values of gold hyperaccumulation than at levels of 0.5 g NaSCN/kg.

As well as thiocyanate, all of the other chemicals trialed resulted in hyperaccumulation of gold. KBr induced the least hyperaccumulation with a maximum value of 17 mg Au/kg dried biomass recorded for the chicory plant, while NaSCN, KI, and $(NH_4)_2S_2O_2$ gave maximum values of 31, 41, and 52 mg Au/kg dried biomass respectively in chicory (Table 3).

KCN gave the highest levels of hyperaccumulation in all of the trials. However, its use as a soil additive is unlikely to be acceptable due to its toxicity.

Thiosulfate gave good accumulation in both *B. juncea* and chicory. While thiosulfate has been used in limited trials elsewhere, it does not often give as good results as thiocyanate, and it has been proposed that the ability of thiosulfate and thiocyanate to solubilise gold is pH dependent [10]. It has been suggested that the ability of some plants to exude acid from root hairs may have an effect on soil pH adjacent to the roots and therefore affect gold uptake [9]. Thiocyanate species are thought to exist in acidic soil conditions [11] and thiosulfate in basic conditions [12]. This could explain why thiosulfate gave better uptake in chicory and slightly worse uptake in *B. juncea* that thiocyanate. In further trials the pH of the artificial substrate should be measured.

Since the pH of the soil has an effect on gold solubility and mobility, it is also possible that the internal pH of the plant has an effect on the transport of gold within the plant. Plants grown in cyanide-amended soils had a much higher gold concentration in the stems and leaves indicating that gold was translocated to a much larger extent. This enhanced mobility, over that occurring with soils amended with other solubilisers, could be due to a favourable internal pH promoting soluble cyanide-gold complexes.

CONCLUSIONS

B. juncea and *B. coddii* are both capable of hyperaccumulating gold when sodium thiocyanate solution is added to artificial substrate.

B. juncea and chicory are both capable of hyperaccumulating gold when either iodide, bromide, cyanide, thiocyanate or thiosulfate solutions are added to artificial gold substrates.

Cyanide addition may induce higher gold concentrations in the leaves and stems of *B. juncea* and *B. coddii* than in the roots in some cases. For all cases of thiocyanate addition the root concentrations were higher. This difference in translocation ability between cyanide and thiocyanate may be due to the effects of the plant's internal pH on the complexes formed.

Thiosulfate was better at inducing hyperaccumulation in chicory than in *B. juncea*. Some plants are able to decrease soil pH in the area adjacent to their roots, making the conditions more favourable for thiocyanate and this may explain the difference in hyperaccumulation.

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