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Gold uptake by perennial ryegrass: The influence of humates on the cycling of gold in soils

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Abstract. An experiment in which perennial ryegrass (*Lolium perenne* L.) was grown in solution cultures containing various forms of radioactive gold (^{198}Au) is described. Uptake of labelled Au from solutions containing either Au chloride or various Au-humic acid (HA) complexes is compared. In each case concentrations of Au in the plant roots were several fold higher than in the plant shoots. However, the Au concentration in roots from some of the Au-HA preparations was only about 5% of that from the Au-HA solutions. High voltage electrophoresis studies indicate the presence of several Au-HA complexes in the solution cultures. The relevance of these findings to the cycling of Au within soils is discussed.

Introduction

Several studies have pointed to the influence of naturally-occurring organic substances (often categorised as humic and fulvic acids) on the form and behaviour of metals in natural systems. Metal complexation is due to the presence of active metal binding sites on the surface of humic (HA) and fulvic (FA) acids, and these have been characterised by several workers (Chen et al. 1978; Murray & Linder 1984; Raspor et al. 1984; Schnitzer 1986). Metal-HA and -FA adsorption processes have also been investigated (Kerndorff & Schnitzer 1980; Skogerboe & Wilson 1981).

Gold is known to be relatively immobile in soils and does not readily enter the aqueous phase (Curtin et al. 1970). It has, however, been reported that Au may be solubilised from minerals and soils by microbial activity (Korobushkina et al. 1983) and by cyanogenic plants (Lakin et al. 1974; Girling & Peterson 1978). Solubilisation of Au by organic acids has also been reported (Freise 1931), but these findings have not been satisfactorily repeated (Fetzer 1946; Owen & Jones unpubl. data). HA can dissolve, complex and transport Au in soils (Baker 1978) while the role of organic matter in determining the biogeochemical distribution of U is discussed by Manskaya

& Drozdova (1968). As long ago as 1937 Goldschmidt (1937) reported the enrichment of Ag and Au in the soil humus layer of an ancient, undisturbed beech and oak forest and in a later discussion of the geochemistry of Au he concluded that enrichment of Au and Ag in the soil surface layers was evidence for their circulation in living plants (Goldschmidt 1954). Malyuga (1964) expanded this concept by proposing that once inside the plant, non-essential metals, such as Au and Ag, would become organically bound and transported to protective and structural tissues or organs, which would later be discarded by the plant (e.g., leaves, rootlets). With the decomposition of the stems, leaves and dead roots the metals, together with the most stable organic compounds would remain in the humus layer. Consequently, the analysis of soil humus has been advocated as a useful technique when prospecting for precious metals (Banister 1970; Curtin et al. 1968, 1971; Curtin & King 1986; Dunn 1986; Hoffman & Booker 1986). There have been several other indications of a strong affinity between Au and Ag and the organic component of soils and these have been discussed elsewhere (Gregoire 1985; Jones et al. 1986). In this paper we report on an experiment undertaken to investigate the influence of HA on gold uptake by perennial ryegrass (*Lolium perenne* L.), and discuss these findings in relation to the possible role of HA in controlling the biogeochemical cycling of Au.

Materials and methods

The experiments described here were performed using a commercially available humic acid extract (Aldrich Chemical Company). This decision was taken because of the long extraction and preparation time required to obtain clean HA/FA fractions from soil (notably in removing the clay content) and because some degree of repeatability and uniformity can be attained by using a single supply of HA. It was recognised that there may be limitations in the use of commercial HA for this type of work (Malcolm & MacCarthy 1986).

Preparation of ^{198}Au compounds

The istope ^{198}Au (half-life, 2.7 days) was produced by irradiating stable gold foil in a flux of 1 x 10^{12} neutrons cm^{-2} s^{-1} for 7 h. This yielded an activity of ca. 1 mCi per mg Au foil. The foil was dissolved in a few drops of aqua regia to produce ^{198}Au chloride, and subsequently diluted to produce a stock solution from which the Au-HA mixtures were prepared. Eleven treatments were prepared in total, consisting of combinations of two final ^{198}Au concentrations (1 and 10 μg l^{-1}), two pH regimes (pH 4.0 and 7.5), two HA concentrations (10 and 100 mg HA l^{-1}) and filtered and unfiltered (0.8 μm Millipore filter) HA solutions, as well as the Au chloride treatment (see Table 1). After preparation, each solution was placed on a separate magnetic stirrer overnight to equilibrate. Each was then adjusted to give identical counts minute^{-1} (at the two concentrations). Adsorption loss on to the glassware overnight was tested and found to be negligible.

Growth and preparation of plants

Seedlings of *Lolium perenne* (L.) were grown from seed, selected for uniformity and cultured in Arnon-Hoagland nutrient solution under controlled environmental conditions until they were 3 weeks old. The plants were grown in aerated, darkened 1 l pots, with 10 plants per pot. The pots were numbered and selected for each treatment randomly with 3 pots per treatment; they were positioned in the growth chamber using the randomised-blocking technique.

Treatment of plants

The plants were cultured in the appropriate Au solution for 48 h, then transferred to the non-active nutrient solution medium for a further 24 h. At the end of the experiment roots were desorbed with carrier Au. The HA extracts tend to cling to the roots, which could give misleading dry weight and root activity measurements. This problem was overcome by briefly placing the roots into an ultrasonic shaker and by copious rinsing with distilled water.

After desorption and decontamination the plants were immediately separated into root and shoot sections, then assayed for ^{198}Au on a Packard 500c Auto-Gamma counting system. Harvested plant material was subsequently dried at 80 °C for 24 h and weighed.

Final values are expressed as Au concentration. Transport index values have also been calculated as a measure of metal transport.

$$\text{Transport index } (\%) = \frac{\text{metal content of shoot} \times 100}{\text{metal content of whole plant}}$$

High voltage electrophoresis

^{198}Au-HA, and ^{198}Au chloride solutions prepared for the plant uptake studies were also electrophoresed at pH 5.3 for 10 min. in pyridine-acetate buffer and the dried electrophoretogram cut in 1 cm paper strips at 90° to the direction of ionic migration. Radioactivity was determined by the counting procedures described above.

Results and discussion

The ^{198}Au and HA concentrations used in this experiment were selected to be representative of those encountered in the field. Au has been reported at 0.02 - 0.12 $\mu g\ l^{-1}$ in leachates extracted from Au bearing mull (Curtin et al. 1970). As might be expected, it is somewhat lower than soluble plus exchangeable Ag concentrations measured in contaminated soils (Jones et al. 1984). The concentrations of 1 and 10 $\mu g\ ^{198}Au\ l^{-1}$ used here had to be somewhat higher to ensure reliable counting statistics. Reported values for organic matter concentrations in soil solutions range from about 1 mg l^{-1} to 1600 mg l^{-1} (Burch et al. 1978).

Forms of Au in the experimental solutions

The electrophoretograms for ^{198}Au chloride and ^{198}Au-HA solutions are shown in Fig. 1. These indicate that several forms of ^{198}Au are present in the HA solution, each with different charge/mass characteristics. However, a high proportion of the ^{198}Au appears to be present in a similar (ionic) form to that in the $^{198}AuCl_4$ solution. The ^{198}Au chloride profile is similar to that reported for the nutrient solutions used by Girling & Peterson (1978). The ^{198}Au-HA electrophoretogram suggests both complexed and ionic forms are present. ^{198}Au is probably complexed with different molecular weight fractions; 9.5% and 8.0%, respectively, of the ^{198}Au activity in the filtered solutions of 10 mg HA l^{-1} and 100 mg HA l^{-1} was retained on the 0.8 μm Millipore filters (2.5 l filtered). Studies with size fractionation permeation gels have suggested ^{198}Au retention with a range of molecular weight components (Jones & Peterson, unpubl. data). Other workers have reported the occurrence of fine ($< 0.05\ \mu$m) gold colloids in soil solutions (Curtin et al. 1970; Ong & Swanson 1974).

Plant uptake studies

The uptake of Au by *Lolium perenne* under the various treatments is shown in Table 1. The mean and standard deviation for each treatment has been calculated by pooling the data from all thirty plants. For each form of Au accumulation in the roots (relative to the shoots) predominated and confir-

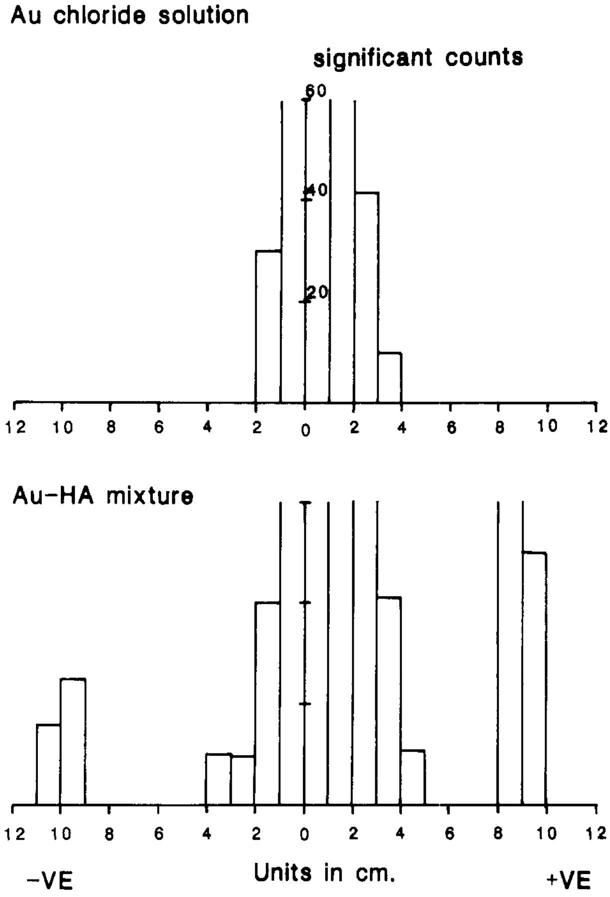

Fig. 1. High voltage electrophoretograms prepared from ^{198}Au solutions.

med the findings of previous workers (Shacklette et al. 1970; Girling & Peterson 1978). However, the uptake varied significantly between treatments. Uptake from the Au chloride solution was the highest of any of the treatments; uptake from HA solutions was dependent on concentration; 100 mg HA l^{-1} significantly reduced uptake. Uptake from 10 mg HA l^{-1} was greater at pH 4.0 than at pH 7.5, whilst the converse was true with 100 mg HA l^{-1} present. In addition, uptake from the filtered HA solutions was less than from the unfiltered samples, particularly with 10 mg HA l^{-1} present. The TI values varied little with treatment. This is different to the situation observed for ^{110m}Ag uptake, where transport to shoots was greater in HA and FA treatments (Jones & Peterson 1986).

Table 1 indicates that adsorption and retention of Au by humates may limit its biological availability and ultimately its rate of recycling within the

Table 1. Uptake of various forms of labelled Au by *Lolium perenne* (L) in 48 hours.

Treatment	Solution ^{198}Au(μg l^{-1})	Solution HA(mg l^{-1})	pH	cpm	Transport Index (%)	Uptake as % of that in $AuCl_4$
Au chloride	10	–	4.0	R 47139 ± 19046 S 1148 ± 613	2.4	100
Au-HA	10	10	4.0	R 23959 ± 10596 S 629 ± 400	2.6	51
Au-HA	10	100	4.0	R 3451 ± 1965 S 90 ± 45	2.5	7.3
Au-HA	10	10	7.5	R 12582 ± 5330 S 224 ± 75	1.7	26.5
Au-HA	10	100	7.5	R 4810 ± 1972 S 100 ± 24	2.0	10.2
Au-HA	10	10	4.0	R 3468 ± 1768 S 74 ± 35	2.1	4.2
Au-HA	10	100	4.0	R 1970 ± 600 S 68 ± 11	3.3	4.4
Au-HA	1	10	4.0	R 2111 ± 1530 S 37 ± 9	1.7	4.4
Au-HA	1	100	4.0	R 396 ± 158 S 6 ± 2	1.5	0.8
Au-HA	1	10	7.5	R 568 ± 406 S 7 ± 3	1.2	1.2
Au-HA	1	100	7.5	R 591 ± 139 S ND		1.2

soil system. Strong adsorption or complexation of Au by humic colloids with possible incorporation into the structure of humic components (Boyle 1979) or the formation of relatively stable organic chelate complexes would thus prevent its subsequent removal from the upper horizons, and lead to the accumulation of Au in soil organic horizons, such as forest litter and mull humus. Clearly, this points to the potential of assaying soil humus for Au and pathfinder elements in relation to biogeochemical prospecting studies (Curtin & King 1986; Dunn 1986; Hoffman & Booker 1986).

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