Extraction and Colorimetric Determination of Picloram in Soil

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A colorimetric method has been developed for estimation of picloram (4-amino-3,5,6-trichloropicolinic acid) in the soil. It involves diazotization of picloram with nitrite in sulfuric acid solution. The color developed is stable in the absence of ultraviolet radiation without a coupling agent; the reaction is specific and not subject to interference from substances commonly present in the soil. Picloram can be quantitatively extracted from most soils by 1*N* NH_4OAc, 2*N* KCl, or a KCl-KOH solution. This method is simple, rapid, and suitable for use in analyzing a large number of samples. It is not sensitive enough for determination of trace quantities of picloram (<0.5 p.p.m.).

Picloram (4-amino-3,5,6-trichloropicolinic acid) is a herbicide which is uniquely effective in controlling many perennial weeds, poisonous plants, shrubs, and other woody species. Several methods for estimating picloram in soils or applicable for this purpose have been devised; these include the use of bioassay techniques (Goring *et al.*, 1965; Grover, 1967; Hamaker *et al.*, 1963; Herr *et al.*, 1966; Leasure, 1964); radioisotope tracing techniques (Hamaker *et al.*, 1966; Meikle *et al.*, 1966); infrared spectroscopy (Ramsey, 1967); thin-layer chromatography (Whitenberg, 1967); and gas chromatography (Bjerke *et al.*, 1967; Hance, 1967; Leahy and Taylor, 1967; Merkle *et al.*, 1966; Saha and Gadallah, 1967; Woolson and Harris, 1967). These methods are extremely sensitive in detecting pure picloram. However, no satisfactory procedure is at present available for quantitative extraction of picloram from the soil. Because of the difficulties involved in extraction of picloram from the soil and in clean-up of the extract required for analysis, suitability of these existing methods for estimation of picloram in the soil is not known. In addition, all the cited methods employ very time-consuming procedures.

In many studies which involve a large number of analyses but do not require extreme sensitivity in detection, such as those on picloram movement and distribution under various conditions in soils or picloram transformations in laboratory experiments, a rapid method for picloram estimation can be employed with advantage. A rapid colorimetric method has now been developed and in use for some time in this laboratory, and has been shown to be satisfactory for many purposes. The colorimetric method devised involves diazotization of the amino group on the picloram molecule with nitrite in an acid medium. The intensity of the bright yellow color developed is measured spectrophotometrically.

EXPERIMENTAL

Apparatus and Reagents. Routine colorimetric measurements were performed on a Spectronic 20 Colorimeter-Spectrometer, using matched Hycel cuvettes for samples. The spectral data presented in Figure 1 were obtained using a Spectronic 600 Recording Spectrophotometer. Sensitivity studies were also performed on a Beckman DU Spectrophotometer.

Analytical grade of 4-amino-3,5,6-trichloropicolinic acid (Tordon acid) was supplied through the courtesy of The Dow Chemical Company. The acid was dissolved in an equivalent amount of KOH to form a potassium salt (pH 7). This salt solution is designated as picloram. Standard picloram solutions were made up from stock by successive dilution.

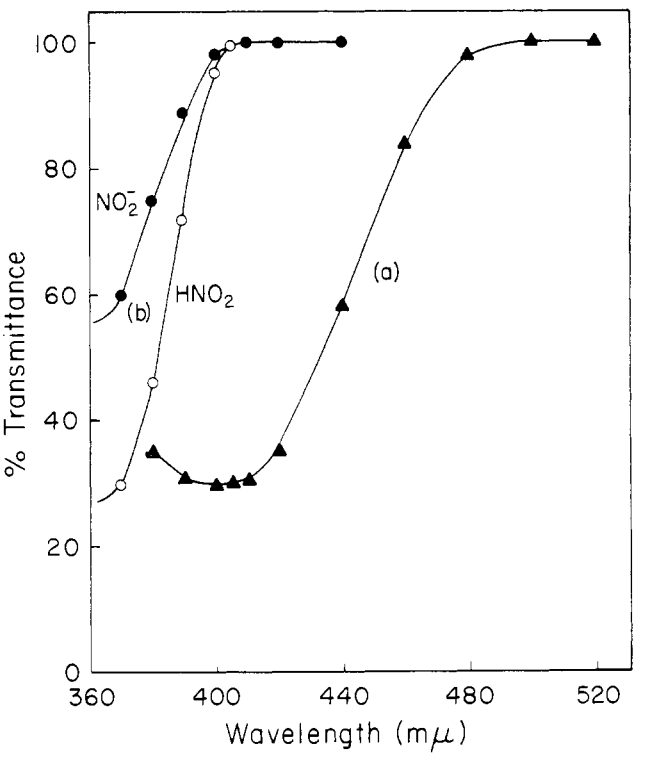

Figure 1. (*a*) **Absorption spectrum of diazotized picloram (20 p.p.m.) developed in H_2SO_4**
(*b*) **Absorption spectra of nitrite (NO_2^-) and nitrous acid (HNO_2)**

Development of Method. The yellow color developed by diazotization of picloram with nitrite in an acid solution exhibits a broad absorption peak between 400 and 410 mμ with the maximum absorption occurring at 405 mμ (Figure 1). A plot of absorbance at 405 mμ *vs.* picloram concentration showed a straight line over the concentration range of 0.5 to 20 p.p.m. (or μg. picloram per ml. solution).

Color Stability. The color developed by diazotization is not stable under day light, fluorescent light, or ordinary incandescent light. Effort in finding a coupling agent to stabilize the diazo color was unsuccessful due mainly to interference caused by color-producing reactions between effective coupling agents and the excess nitrite present in test solutions. Treatment of test solutions with sulfamic acid eliminated nitrite, but also destroyed part of the color com-

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plex. However, the color was completely stable in the absence of ultraviolet radiation even without a coupling agent. The color complex was stable at all wavelengths in the visible range and when the colored solution was stored in the dark or in a room lighted only with a yellow-colored light bulb, no change in absorbance reading was observed even after 24 hours.

COLOR INTENSITY. The intensity of the diazo color, as measured by absorbance at 405 mμ, is affected by the kind and strength of the acid medium in which the color is developed. Figure 2 shows that sulfuric acid is superior to other acids as the medium for developing the most intense color at each given acid concentration. Although picloram also developed good color in the nitric acid medium, the acid itself is often colored and can interfere with the determination. Hydrochloric acid provided the most erratic results. At very high concentrations of phosphoric acid (>16*N*), intensity of the color developed approached that developed in sulfuric acid at 3–9*N*, whereas when the strength of sulfuric acid was increased to 12*N* and above, the absorbance values became erratic. In all cases, the maximum color intensity develops in less than 10 minutes.

BACKGROUND ABSORPTION. Sulfuric acid shows no absorption in the region used for measurement of picloram color. Nitrite absorbs strongly below 400 mμ either as an ion in a neutral salt solution or as an acid in the sulfuric acid medium. The acid form shows stronger absorption peaks than the ionic form of nitrite (see Figure 1, *cf.* Wright, 1914). In the region of picloram absorption peak, the nitrite absorption edge drops sharply to nil. At 405 mμ, the absorptivity of nitrite ion and nitrous acid is the same, and their interference to picloram color estimation is almost negligible. To eliminate the nitrite interference completely, nitrite, should be present in the blank sample used for zeroing the spectrometer. For determination of picloram in a sample, a valid blank would be taken from a comparable check sample containing no picloram. Thus the sample and the blank can be treated in the same manner with sulfuric acid and nitrite solutions. If no comparable check sample is available, the blank should consist of an aliquot of the picloram-containing sample plus nitrite but without sulfuric acid.

PRESENCE OF FOREIGN SUBSTANCES. The diazotization reaction between picloram and nitrite in sulfuric acid solution is specific. Although soil extracts are often colored, they usually do not contain any substance which adds color during the diazotization process or interfere with picloram estimation when proper blanks are used. Tests on estimation of picloram added to various soil extracts, including a highly colored muck extract, show that no interference was found. Nor does the color development seem to be interfered by the presence of any foreign ions. A large number of ions was tested, including sodium, potassium, lithium, ammonium, magnesium, manganese, zinc, copper, nickel, iron, aluminum, chloride, nitrate, phosphate, borate, acetate, citrate, arsenate, molybdate, and silicic acid. Excess calcium and barium will precipitate in the sulfuric acid solution. Carbonate will decompose in acid, but does not interfere with the picloram color development.

EXTRACTION FROM SOIL

Picloram exists as an organic anion and tends to be sorbed by the organic matter and the amorphous inorganic materials of the soil (Hamaker *et al.*, 1966). It is highly soluble in water as a salt and sufficiently soluble as an acid (around 430 p.p.m.). Existing procedures for extraction of picloram in soil involve either the use of an organic extractant under very acid conditions or dilute aqueous alkali. Since the procedure developed requires color development in aqueous media, organic extraction is not suitable. Dilute KOH can remove picloram quantitatively from the soil, but will also remove a large amount of other organic matter, thus posing a difficult task of cleaning up the extract for analysis.

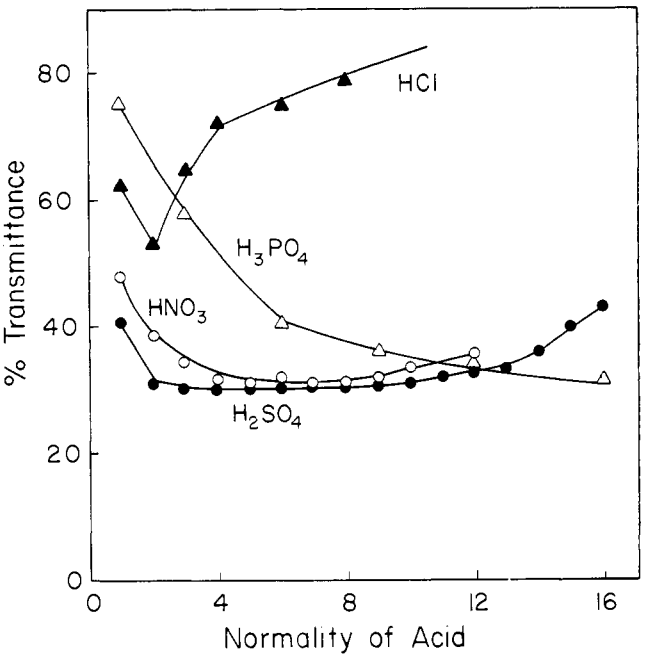

Figure 2. Intensity of diazotized picloram (20 p.p.m.) color as affected by kind and strength of acid used for development medium

Trials with various extraction solutions have shown that picloram can be quantitatively extracted from the soil with little removal of organic matter by using a solution containing a high concentration of salt, *e.g.*, 2*N* KCl. A slightly buffered solution, such as 1*N* NH_4OAc, is also efficient in extracting picloram from the soil. More strongly buffered solutions or solutions of higher pH (*e.g.*, K_2HPO_4–KH_2PO_4 solutions) remove too much organic matter and cause the extracts to become too colored for colorimetric analysis. Recovery studies were performed on soils containing 1 to 25 p.p.m. of picloram. Soils representing a wide range of texture, organic matter content, and pH differences were tested for picloram recovery. The picloram in the soil was extracted by shaking the soil with the 2*N* KCl or 1*N* NH_4OAc solution at a 1-to-10 soil-to-solution ratio for 30 minutes; after allowing the soil to settle, an aliquot of the clear supernatant liquid was pipetted off directly for colorimetric analysis, with another aliquot used as blank. The extraction efficiency, as measured by the percentage of picloram recovered, generally decreased with increasing organic matter content and decreasing pH of the soil. The percentage of picloram recovery was the same irrespective of the picloram concentration in the soil. Table I shows the average picloram recoveries as influenced by soil properties.

Quantitative recovery of picloram was obtained from soils containing less than 3% organic matter (1.5% C) and pH above 6. For soils containing more than 3% organic matter and with pH below 6, recovery of picloram by 2*N* KCl is sometimes less than complete. Using 2*N* KCl extraction, picloram recovery was 80 to 84% from unlimed samples of Puyallup and Winlock soils, which are highly acidic and have

Table I. Influence of Soil Properties on Picloram Recovery from Representative Soils by Using 2*N* KCl as Extracting Solution

Soil	Cation Exchange Capacity, me./100 g.	Silt, %	Clay, %	Organic Matter,[a] %	pH, Paste	Picloram Recovered by 2*N* KCl %
Ephrata sandy loam	8.2	32	8	0.7	7.2	100
Ritzville silt loam	9.8	62	11	0.8	6.7	100
Cusick clay, B horizon	21.5	41	54	1.0	6.4	100
Walla Walla silt loam	13.4	62	15	1.8	6.1	98
Palouse silt loam	19.0	61	27	3.0	5.9	95
Helmer silt loam	18.9	65	14	6.3	5.1	92
Hesson clay loam	14.3	49	31	15.4	4.9	85
Hoquiam silty clay	5.0	47	44	10.3	4.8	65
Viola silty clay loam	26.6	63	34	5.3	4.6	50
Kinney silty clay	22.1	40	50	24.9	3.9	35

[a] The % organic matter values were estimated by multiplying the % carbon content by 2.

Table II. Influence of Soil pH Adjustment on Picloram Recovery from Representative Soils by Using 2*N* KCl as Extracting Solution

Soil	Clay, %	Organic Matter, %	pH, Paste		Picloram Recovered by 2*N* KCl, %
Puyallup silt loam	25	6.8		5.1	80
			Limed to	7.1	95
Winlock silty clay loam	40	5.9		5.1	84
			Limed to	7.3	97
Artic clay, B horizon	64	1.5		4.6	16
			KOH adjusted to	7.0	100

high organic matter content (see Table II). However, after treating the soil with lime and incubating it for one month to adjust the soil pH to 7, picloram recovery from these limed samples of Puyallup and Winlock soils by 2*N* KCl was nearly complete. Similarly, when the unlimed soils were extracted with a 2*N* KCl solution containing sufficient KOH to adjust the soil pH to 7, picloram could also be recovered quantitatively. It appears that soil pH, rather than the organic matter content *per se* exerts a greater influence on the efficiency of picloram extraction from soil. Extractants maintaining soil pH at 7 and possessing sufficient ionic strength seem to be efficient in removing picloram from soil.

The choice between using the KCl or the NH_4OAc solution as extractant will depend on pH of the soil and color of the soil extract. The NH_4OAc extract is more colored than the KCl or the KCl–KOH extract. Occasionally, a soil is not buffered to above pH 6.1 by the NH_4OAc solution, and picloram recovery would not be quantitative. On the other hand, adjusting the soil pH to 7 with KOH before KCl extraction can be quite time-consuming. However, when the amount of KOH required for pH adjustment of a given soil is known, subsequent extractions can be carried out more speedily. Regardless of the extractant used, efficiency of picloram recovery was unchanged whether picloram was added to the soil immediately before extraction or added to the soil and incubated at slightly below $-1/3$ bar water potential and temperature just above freezing for as long as three months before extraction. Analyses on these stored samples were performed at weekly intervals.

PROPOSED METHOD

Picloram in the soil is extracted with either 1*N* NH_4OAc, 2*N* KCl, or a 2*N* KCl solution containing sufficient KOH to make the extract pH 7. Add the extractant solution to the soil at 1-to-10 (w. per v.) soil-to-solution ratio, and shake the soil–solution mixture mechanically for 30 minutes. Allow the soil to settle or centrifuge down the soil. Aliquots of the clear supernatant liquid can be taken directly without filtration for colorimetric analysis.

The colorimetric analysis must be carried out in a room free of ultraviolet radiation. This can be conveniently done in a closed room lighted only with a yellow-colored light bulb. The amount of the extract sample to be used for colorimetric analysis depends on the concentration of picloram in the sample. If a 1-ml. sample is used, add 5 ml. of 7*N* H_2SO_4; if a 5-ml sample is used, add 1 ml. of concentrated H_2SO_4. Place the sample and H_2SO_4 in a matched Hycel cuvette (19 × 150 mm.). Add 1 ml. of 0.1*M* $NaNO_2$. Mix the solution in the cuvette thoroughly (*e.g.*, by using a Vortex shaker). Let the solution stand for approximately 10 minutes to allow color to develop fully before reading the color absorbance at 405 mμ on a spectrometer. The total volume of 7 ml. is convenient for analyzing the color solution in the cuvette on a Spectronic 20 spectrometer. If another instrument is used requiring special cuvettes, the solution can be mixed in an ordinary test tube and transferred to the proper cuvette after color development. For the absorbance or transmittance reading, compare each sample with its proper blank, the transmittance of which should be set at 100. Estimate the picloram concentration in the sample from a standard calibration curve. A typical standard curve, using 5-ml. samples, shows 93% transmittance at 1 p.p.m. picloram, 73% at 5 p.p.m., 55% at 10 p.p.m., and 30% at 20 p.p.m.

COMMENTS

The advantages of the proposed colorimetric method are that the procedure is simple and rapid, no special instrument is required, and a large number of samples can be handled with ease. For instance, for studying the movement of picloram through a soil column under leaching conditions, results can be obtained within minutes after the eluent comes off the column into the fraction collector. To analyze the

two-hundred fractional samples by a gas chromatographic procedure would require several days.

Using 5-ml. samples, the proposed method is sensitive enough to detect 0.5 p.p.m. of picloram in the extract. This method is not sufficiently sensitive for trace analysis. By modifying the procedure, such as by adopting a narrower soil-to-solution ratio for extraction, concentrating the extract, employing larger amount of sample and smaller amount of more concentrated acid and nitrite solutions, or making use of the trace analysis method by Reilley and Crawford (1955), the sensitivity of this method can be improved. The disadvantages of these modifications are that additional steps in the procedure are required and that the background color may be increased to becoming a limiting factor in analysis.

Since both the radioisotope tracing and the gas chromatographic methods involve the more active carboxyl group on the picloram molecule for detection, whereas the colorimetric method involves the more stable amino group, these methods could be used in supplement for study of picloram breakdown.

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