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# Influence of Soil Properties on the Phytotoxicity of Atrazine, Ametryne, Prometryne, and Diuron In Puerto Rican Soils

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## INTRODUCTION

Initial phytotoxicity of 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (Atrazine), 2-methylmercapto-4-ethylamino-6-isopropylamino-s-triazine (Ametryne), 2-methylmercapto-4-6-bis-isopropylamino-s-triazine (Prometryne), and 3-(3,4-dichlorophenyl-1,1-dimethyl urea) (Diuron) has been reported to vary with different soil types (*7,13,14,15,17,18*).[2] In Puerto Rico, these soil-applied herbicides have been used extensively for weed control in crops such as sugarcane, pineapple, and coffee. Since the Island has a nearly uniform tropical temperature, it is natural to expect a few different soil series. However, owing to great differences in rainfall, relief, vegetation, and parent rock, there are 358 soil types, phases, and miscellaneous land types recognized in the Island (*12*). Therefore, a study was undertaken to determine the extent to which variations in organic matter, cation exchange capacity, exchangeable bases (Ca, Mg, and, K), soluble phosphorus, pH, and texture, as exemplified by soil types, interfered with the phytotoxic action of the above-mentioned herbicides. Knowledge of the relationship or interaction of phytotoxicity to soil properties should aid in establishing safe, effective rates for these herbicides. There is no published information as yet on the behavior of Atrazine, Ametryne, Prometryne, or Diuron in the soil under tropical conditions.

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2 Italic numbers in parentheses refer to Literature Cited, pp. 279–80.

## REVIEW OF LITERATURE

Sheets (*13*), after studying the comparative toxicities of four urea herbicides using 17 different soil types in the greenhouse, reported that the $ED_{50}$[3] values of the herbicides referred to above were positively correlated with such soil properties as cation-exchange capacity, percentage organic matter, and clay. Organic matter seemed to be, among the other soil factors, the most important one in ameliorating the toxic effects of the substituted urea herbicies, as shown by the difference in growth of the indicator plants. This same investigator and his coworkers (*14*) in a subsequent study also found that Simazine $ED_{50}$ value was positively correlated with both organic matter and cation-exchange capacity, and negatively so with the pH of the soil.

Results obtained by Upchurch (*17*) in an experiment intended to determine the influence of various soil factors upon the relative toxicity of Diuron revealed that organic matter and cation-exchange capacity of the soils involved were highly correlated with a reduction in growth of experimental plants. On the other hand, correlations between Diuron $ED_{50}$ values and clay content, base saturation, soluble phosphorus, and pH were not significant. In another series of investigations Upchurch *et al.* (*18,19*) fully corroborated the above-mentioned findings.

Similarly in a study pursued by Dubey and Freeman (*5*) on the phytotoxic action of Linuron and Diphenamide it was concluded that their $ED_{50}$ values were directly correlated with soil organic matter. Moreover, several other investigators (*3,6,7,10*) in their efforts to elucidate the influence exerted by the chemical, as well as physical properties of the soil upon the phytotoxicity of herbicides, have singled out organic matter as the most important of them all.

Most of the information reviewed above has been gathered under temperate and subtropical conditions. Very little is known about the phytotoxic action of herbicides in tropical soils such as those of Puerto Rico.

## MATERIALS AND METHODS

Thirteen soil types, representing a wide range of chemical and physical properties, were collected from various regions of Puerto Rico. These were brought into the greenhouse in large galvanized iron deposits, where they were, soon after, sieved, air-dried, and ground. The air-dry moisture content and field capacity were determined.

Five hundred-gram portions of the above-processed soil types were weighed and filled into unperforated No. 2 tin cans. Four herbicides, namely: Atrazine, Ametryne, Prometryne, and Diuron, were applied to the soil

[3] The $ED_{50}$ value is equivalent to that dosage of a herbicide capable of reducing the fresh weight of oat plants by 50 percent.

media at concentrations of 0.0, 0.5, 1, 2, 4, 8, 16, and 32 ppm., except in case of the Caño Tiburones soil. In view of the naturally high organic-matter content of this soil, two additional concentrations, 64 and 128 p.p.m., were used. Stock solutions of each herbicide were prepared from 80W (80-percent wettable powder) formulations. The herbicide concentration corresponding to each tin can was drawn from the stock solutions by means of a pipette, and suspended in water, so that when applied to the soil, this would be wet to field capacity. The herbicide suspensions were not directly applied to soil-culture surfaces. Instead, the herbicides were combined with the soils by alternately adding one-third of soil and one-third of the herbicide to the tin can. All concentration series were run in triplicate and arranged according to the randomized block design.

After the herbicides were added to the soil cultures 13 oat seeds (*Avena sativa* L., Kanota) were planted per can by pressing the embryo end vertically halfway into the soil. The Kanota oat seed was obtained from Dr. Wm. H. Harvey of the Agricultural Extension Service, University of California, at Davis, Calif., to whom the authors are indebted. The cans were watered daily and brought to field capacity periodically. After a 4-week growing period in a greenhouse bench, 10 oat plants were cut off from each can at ground level. Their fresh weights were recorded and afterwards used for calculating the $ED_{50}$ values.

Mechanical analysis was made on each soil in duplicate, according to the hydrometer method of particle-size distribution as described by Day (*4*). The chromic acid oxidation method of Peech *et al.* (*11*) was employed to determine organic-matter content. The ammonium acetate leaching method developed by Jackson (*9*) was followed in the determination of cation-exchange capacity. The amount of calcium and magnesium present in the various soils was detected by the Versenate method described by Cheng & Bray (*2*), except that Calcein was used as Ca indicator, and Calmagite as Ca and Mg indicator. Potassium was determined photometrically with a Coleman Universal Spectrophotometer attached to a flame photometer. Determinations of organic matter, cation-exchange capacity, exchangeable calcium, magnesium, and potassium were made in quadruplicate for each soil type. Soluble phosphorus was determined colorimetrically using ammonium molybdate and 1-amino-2-naphthol 4-sulfonic acid after extracting with Morgan's Universal Extracting Solution. The pH of each soil was determined potentiometrically with a Beckman pH-meter using 1:1 soil-water mixture. The resulting physical and chemical properties of the 13 selected soil types are listed in table 1.

The $ED_{50}$-value method was adopted to evaluate the effect of soil types and chemical treatments on the growth made by the aereal parts of the indicator plants after Dubey and Freeman (*5*). Thus, $ED_{50}$ values were calculated for each replication under the various treatments as follows:

The fresh weights of shoots as percentage of controls were plotted against the logarithm of the herbicide concentration. A free-hand curve was traced by connecting the resulting points. The value on the chemical concentration axis, which corresponded with the point of intersection on the curve, and the 50-percent shoot growth represented the $ED_{50}$ value as a logarithm. The antilogarithm of this value was equivalent to the actual $ED_{50}$ value in p.p.m.w. (parts per million by weight). Analyses of variance and the least-significant difference test were performed to the actual $ED_{50}$ values of the herbicides for the different soil types. Correlations and regression analyses were made on the data by the Statistical Section of this Station.

TABLE 1.—*Chemical and physical properties of 13 soils*

| Soil type | Organic matter | Cation exchange capacity | Exchange bases | | | Soluble phosphorus | pH | Texture | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ca | Mg | K | | | Sand (2–0.05 mm.) | Silt (0.05–0.002 mm.) | Clay (<0.002 mm.) |
| | *Percent* | *meq./100 g.* | *meq./100 g.* | *meq./100 g.* | *meq./100 g.* | P.p.m | | *Percent* | *Percent* | *Percent* |
| Alonso | 3.2 | 13.8 | 5.4 | 2.0 | 0.91 | 4.0 | 5.1 | 14.9 | 39.3 | 45.8 |
| Bayamón | 1.7 | 5.0 | 2.7 | 1.5 | .19 | 6.0 | 4.7 | 68.1 | 4.4 | 27.5 |
| Caño Tiburones | 36.0 | 86.0 | 61.9 | 14.0 | .80 | 6.0 | 5.5 | 36.0 | 36.0 | 28.0 |
| Coloso | 3.7 | 23.0 | 14.9 | 5.8 | .15 | 8.0 | 5.7 | 22.7 | 37.4 | 39.9 |
| Coto | 3.2 | 14.0 | 16.6 | 0.7 | .35 | 13.0 | 7.7 | 23.4 | 24.8 | 51.8 |
| Fraternidad | 2.1 | 36.0 | 24.0 | 10.4 | .46 | 15.0 | 6.3 | 15.5 | 32.5 | 52.0 |
| Humata | 1.7 | 10.1 | 2.2 | 0.9 | .27 | 4.0 | 4.5 | 10.1 | 50.9 | 39.0 |
| Mabí | 4.9 | 26.5 | 11.5 | 14.2 | .56 | 3.0 | 5.7 | 22.7 | 40.7 | 36.6 |
| Moca | 3.8 | 31.0 | 23.4 | 4.7 | .22 | 6.0 | 5.8 | 26.3 | 27.7 | 46.0 |
| Múcara | 3.3 | 19.6 | 13.6 | 7.8 | .14 | 6.0 | 5.8 | 28.0 | 47.0 | 25.0 |
| Pandura | 2.0 | 7.7 | 5.5 | 2.4 | .13 | 5.0 | 5.7 | 59.4 | 28.2 | 12.4 |
| San Antón | 2.7 | 26.1 | 23.8 | 5.9 | .58 | 16.0 | 6.7 | 24.3 | 49.7 | 26.0 |
| Toa | 0.6 | 8.0 | 6.2 | 1.4 | .17 | 7.7 | 6.0 | 60.9 | 25.1 | 14.0 |

## RESULTS

Table 2 shows the initial phytotoxicity of Atrazine, Ametryne, Prometryne and Diuron, as influenced by the 13 soil types used in the study, upon the first oat crop. The data thus presented indicate that Atrazine, regardless of soil type, resulted the most toxic herbicide. This is revealed by the fact that, in nearly all instances, the least amount of Atrazine was needed to cut down the fresh weight of oats by 50 percent ($ED_{50}$ value). Apparently Diuron was somewhat more toxic than Ametryne inasmuch as its $ED_{50}$ values were lower in 8 of the 13 soils. The least toxic of the four herbicides was undoubtedly Prometyrne. Sheets (*15*) and Harris (*7*) obtained results similar to those expressed above. However, they did not include Ametryne and Diuron simultaneously in their investigations. As can be seen in table 2, Prometryne seems to be more intensively adsorbed by soils than either Atrazine, Ametryne, or Diuron. Talbert (*16*) and Harris (*8*) have come to the same conclusion. They demonstrated that the Prometryne adsorptive capacity of soils, in general, was much higher than for other triazines and substituted urea herbicides. This may explain the strikingly higher $ED_{50}$ values displayed by Prometryne over the other chemicals.

All chemicals were most harmful to oats in the Toa soil. There was not much variation in the $ED_{50}$ value among them in this respect. On the other hand, Caño Tiburones organic soil had to be supplied with the highest amounts of each herbicide, so that the fresh weight of oats could be reduced

TABLE 2.—*Effect of soil on the phytotoxicity of 4 herbicides*

| Soil type | $ED_{50}$ value (p.p.m.w.) | | | |
|---|---|---|---|---|
| | Atrazine | Ametryne | Prometryne | Diuron |
| Alonso | 0.05 | 0.66 | 1.50 | 0.60 |
| Bayamón | .25 | .68 | 1.99 | .56 |
| Caño Tiburones | 10.70 | 12.83 | 48.67 | 18.40 |
| Coloso | .42 | 1.89 | 4.34 | 1.20 |
| Coto | .04 | .10 | .29 | .43 |
| Fraternidad | .16 | .43 | .99 | .81 |
| Humata | .04 | .58 | 2.28 | .07 |
| Mabí | .57 | 1.56 | 31.33 | 2.27 |
| Moca | .46 | 1.53 | 2.88 | 1.44 |
| Múcara | .14 | .96 | 2.52 | .40 |
| Pandura | .06 | .18 | .28 | .06 |
| San Antón | .57 | 1.11 | 3.31 | 1.76 |
| Toa | .03 | .03 | .05 | .03 |
| L.S.D. 0.05 | 0.51 | 0.77 | 4.19 | 0.88 |
| L.S.D. .01 | .69 | 1.03 | 5.68 | 1.19 |

50 percent. A glance at table 1 shows that Caño Tiburones soil contains the highest percentage of organic matter. Not only that, but it also shows that the cation-exchange capacity and exchangeable bases were high. It is interesting to note that the Prometryne $ED_{50}$ value in the Caño Tiburones soil was about 4 times higher than for Atrazine, Ametryne, and Diuron. The Mabí soil which was second to the above soil in organic-matter content had also an extremely high Prometryne adsorptive capacity. The $ED_{50}$ value of Prometryne in this particular soil was 31.33, while those pertaining to Atrazine, Ametryne, and Diuron were 0.57, 1.56 and 2.27, respectively. It can be attributed to this characteristic the fact that, under field conditions, Prometryne is much more selective than the other three herbicides.

Simple correlation of Atrazine, Ametryne, Prometryne, and Diuron $ED_{50}$ values with the various soil properties included in the study is shown in table 3. $ED_{50}$ values of all four herbicides were positively and significantly correlated with the following soil properties: Organic matter, cation-exchange capacity, exchangeable calcium, and exchangeable magnesium. Of these soil factors, organic matter was the most highly correlated with $ED_{50}$ values for the four herbicides.

These findings are in close agreement with the results obtained by several other investigators (*5,13,15,17,18*). Moreover, the significant positive correlations among the above factors (table 3) indicate that they are interrelated. In fact, organic matter, cation-exchange capacity, exchangeable calcium, and exchangeable magnesium were found to be significantly correlated. On the other hand, the lack of correlation between $ED_{50}$ values for chemicals and percentage of clay is indicative of the little influence exerted by the clay fraction of the soil upon the phytotoxic action of the herbicides.

Results obtained by Sheets (*13*) differ from ours. He found the percentage of clay to be positively correlated with $ED_{50}$ values for several urea herbicides. Dubey and Freeman (*4*) also failed to relate clay content with Linuron or Diphenamid toxicity. They stated that adsorption into the mineral-soil colloids, if it occurs at all, does not affect the phytotoxicity of either herbicides. This is of utmost significance, since the great majority of our soils have a high clay content. Up to the present it was believed by our herbicide people that clay content and herbicide activity were inversely related.

Simple correlations were also calculated, excluding the Caño Tiburones soil. These are presented in table 4. It is apparent that the organic-matter content of soils was still significantly correlated with the $ED_{50}$ values of Atrazine, Ametryne, Prometryne, and Diuron. However, correlations between the $ED_{50}$ values and some of the other soil properties (exchangeable calcium, cation-exchange capacity, etc.) were not significant.

Since organic matter was found to be the most important soil property in affecting the phytotoxicity of the four herbicides, the regression equations $y = -0.6147 + 0.3118X$ for Atrazine; $y = -0.1862 + 0.3618X$ for Ametryne; $y = 0.3951 + 1.3829X$ for Prometryne; $y = -0.5648 + 0.4993X$ for Diuron were used to construct the theoretical relationship between percentage soil organic matter and p.p.m.w. of herbicides required for 50-percent fresh-weight reduction of oats (fig. 1). Using the above relationship, the amount of herbicides required to cause a 50-percent fresh-weight reduction of oats can be predicted through the increasing amount of organic matter under different soil types.

From the regression lines of the four herbicides illustrated in figure 1, the general trend indicates the higher the percentage of increasing soil organic matter, the greater the amount of herbicides is required to cause a 50-percent fresh-weight reduction of oats. It is evident that Prometryne needs the greatest increasing amount, and Atrazine the least in order to give the

TABLE 3—*Simple correlation of soil properties among themselves and with $ED_{50}$ value of 4 herbicides*[1]

| Factors correlated | Organic matter | Cation exchange capacity | Exchangeable bases | | | Soluble phosphorus | pH | Sand | Silt | Clay |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ca | Mg | K | | | | | |
| | *Percent* | *Meq./100 g.* | *Meq./100 g.* | *Meq./100 g.* | *Meq./100 g.* | *P.p.m.* | | *Percent* | *Percent* | *Percent* |
| $ED_{50}$ Atrazine | 0.995** | 0.906** | 0.883** | 0.566* | 0.489 | −0.108 | −0.097 | 0.055 | 0.057 | −0.134 |
| $ED_{50}$ Ametryne | .993** | .920** | .885** | .602* | .482 | −.147 | −.131 | .004 | .098 | −.100 |
| $ED_{50}$ Prometryne | .868** | .818** | .723** | .784** | .534 | −.286 | −.128 | −.052 | .159 | −.078 |
| $ED_{50}$ Diuron | .995** | .926** | .899** | .615* | .526 | −.096 | −.075 | .015 | .080 | −.098 |
| Organic matter (percent) | — | .913** | .885** | .588* | .511 | −.136 | −.081 | .003 | .082 | −.084 |
| C.E.C. | — | — | .971** | .766** | .534 | .088 | .070 | −.216 | .205 | .114 |
| Exch. Ca | — | — | — | .664* | .470 | .249 | .243 | −.156 | .141 | .090 |
| Exch. Mg | — | — | — | — | .416 | .004 | .054 | −.260 | .318 | .070 |
| Exch. K | — | — | — | — | — | .043 | .002 | −.419 | .311 | .305 |
| Soluble P | — | — | — | — | — | — | .747** | −.176 | .014 | .239 |
| pH | — | — | — | — | — | — | — | −.144 | −.000 | .208 |
| Total sand (percent) | — | — | — | — | — | — | — | — | −.721** | −.748** |
| Total silt (percent) | — | — | — | — | — | — | — | — | — | .080 |

[1] * Significant at 5-percent level; ** Significant at 1-percent level.

TABLE 4.—*Simple correlation of soil properties among themselves and with $ED_{50}$ value of 4 herbicides (without Caño Tiburones)*[1]

| Factors correlated | Organic matter | Cation exchange capacity | Exchangeable bases | | | Soluble phosphorus | pH | Sand | Silt | Clay |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ca | Mg | K | | | | | |
| | *Percent* | *Meq./100 g.* | *Meq./100 g.* | *Meq./100 g.* | *Meq./100 g.* | *P.p.m.* | | *Percent* | *Percent* | *Percent* |
| $ED_{50}$ Atrazine | 0.614* | 0.614* | 0.529 | 0.609* | 0.137 | 0.126 | 0.091 | −0.175 | 0.177 | 0.083 |
| $ED_{50}$ Ametryne | .744** | .564 | .363 | .547 | .052 | −.183 | −.174 | −.367 | .315 | .228 |
| $ED_{50}$ Prometryne | .644* | .312 | .010 | .728** | .274 | −.340 | −.076 | −.195 | .220 | .070 |
| $ED_{50}$ Diuron | .742** | .705* | .564 | .717** | .387 | .135 | .184 | −.355 | .243 | .280 |
| Organic matter (percent) | — | .558 | .402 | .593* | .316 | −.162 | .183 | −.530 | .315 | .465 |
| C.E.C. | — | — | .876** | .757** | .263 | .422 | .353 | −.602* | .360 | .525 |
| Exch. Ca | — | — | — | .483 | .120 | .710** | .670* | −.432 | .206 | .428 |
| Exch. Mg | — | — | — | — | .217 | .079 | .129 | −.350 | .347 | .172 |
| Exch. K | — | — | — | — | — | .114 | .060 | −.517 | .330 | .430 |
| Soluble P | — | — | — | — | — | — | .744** | −.169 | .020 | .226 |
| pH | — | — | — | — | — | — | — | −.138 | .005 | .196 |
| Total sand (percent) | — | — | — | — | — | — | — | — | −.726** | −.748** |
| Total silt (percent) | — | — | — | — | — | — | — | — | — | .087 |

[1] * Significant at 5-percent level; ** significant at 1-percent level.

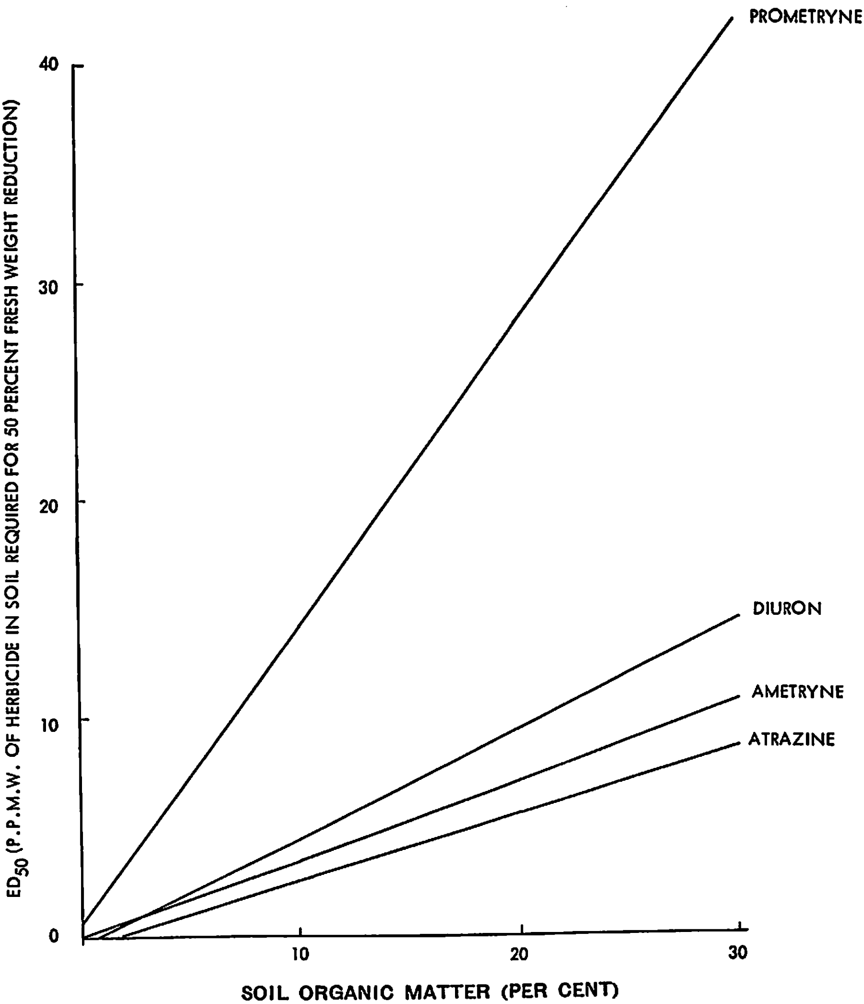


FIG. 1.—Theoretical relationship between percentages soil organic matter and p.p.m.w. (parts per million by weight) of herbicides required for 50-percent fresh-weight reduction of oats.

same fresh-weight reduction. A slightly greater increasing amount of Ametryne than of Diuron is required for soil containing very low organic matter, whereas the reverse is true for soil containing higher organic matter. Although the predicted herbicide dosage based on organic matter lacks the practical field application value, it would certainly aid in determining the relative herbicide rate under a great diversity of Puerto Rican soil types.

Finally, multiple and partial correlations were calculated for those soil properties which showed significant simple correlations with $ED_{50}$ values (table 5). The multiple correlation $R_{y \cdot x_1 x_2}$, representing the combined relation of organic matter, the above-referred-to soil properties, and the $ED_{50}$ values for the various herbicides were significant. When either the cation-exchange capacity, the exchangeable calcium, or exchangeable magnesium was held constant, the partial correlations, $R_{yx_1 \cdot x_2}$, were also significant, except for that between organic matter and the $ED_{50}$ value for Prometryne. On the other hand, when the organic matter was the one soil

TABLE 5.—*Multiple and partial correlation of soil factors with $ED_{50}$ value of 4 herbicides*[1]

| $X_2$ | $R_{y \cdot x_1 x_2}$ | $R_{yx_1 \cdot x_2}$ | $R_{yx_2 \cdot x_1}$ |
|---|---|---|---|
| C.E.C. | 0.995** | 0.974** | −0.067 |
| Ca | .995** | .980** | .060 |
| Mg | .996** | .994** | −.246 |
| | $y$ = *Atrazine* $ED_{50}$ | $x_1$ = organic matter | |
| C.E.C. | 0.994** | 0.959** | 0.274 |
| Ca | .993** | .969** | .116 |
| Mg | .994** | .990** | .198 |
| | $y$ = *Ametryne* $ED_{50}$ | $x_1$ = organic matter | |
| C.E.C. | 0.870** | 0.517 | 0.125 |
| Ca | .873** | .709** | −.194 |
| Mg | .932** | .811** | .682* |
| | $y$ = *Prometryne* $ED_{50}$ | $x_1$ = organic matter | |
| C.E.C. | 0.996** | 0.972** | 0.448 |
| Ca | .996** | .978** | .400 |
| Mg | .996** | .993** | .372 |
| | $y$ = *Diuron* $ED_{50}$ | $x_1$ = organic matter | |

[1] * Significant at 5-percent level; **significant at 1-percent level.

factor held constant, none of the partial correlations ($R_{yx_2 \cdot x_1}$) was significant, with the sole exception of that between exchangeable magnesium and the Prometryne $ED_{50}$ value. This again demonstrates that organic matter was the soil factor more closely related to the phytotoxic action of herbicides.

## SUMMARY

Thirteen soils representing a wide range of physical and chemical properties were used in this study. Four herbicides including Atrazine, Ametryne, Prometryne, and Diuron were applied at a concentration series from 0.5 to 32 p.p.m. to each soil, with the exception of Caño Tiburones soil. Kanota oat (*Avena sativa* L.) was chosen as an indicator plant. $ED_{50}$ values were

obtained for the various soil types. The result indicated that $ED_{50}$ values varied greatly with different soil types. Simple, partial, and multiple correlations were made among $ED_{50}$ values and different soil properties. It was found that the organic matter was the major soil property which contributed chiefly to the phytotoxicity of herbicides. A theoretical relationship between percent soil organic matter and p.p.m.w. of herbicides required for 50-percent fresh-weight reduction of oat was obtained for herbicide dosage-prediction purpose.


## RESUMEN

En este estudio se incluyeron 13 suelos, los cuales tienen una amplia variedad de propiedades físicas y químicas. A cada uno de ellos, excepto el de Caño Tiburones, se le aplicaron cuatro yerbicidas, a saber, Atrazine, Ametryne, Prometryne y Diuron a concentraciones que variaron de 0.5 a 32 p.p.m. Se escogió la variedad Kanota de avena (*Avena sativa* L.) como planta indicadora. Se obtuvieron valores de $ED_{50}$ para los distintos suelos. Los resultados indicaron que hubo una gran variación en los valores de $ED_{50}$, según los diferentes tipos de suelos. Se hicieron correlaciones sencillas, parciales y múltiples entre los valores de $ED_{50}$ y las diferentes propiedades del suelo. Se encontró que, entre las principales propiedades del suelo, el contenido de materia orgánica fue lo que más contribuyó a la fitotoxicidad de los yerbicidas. Para poder predecir la dosis de yerbicida adecuada, se obtuvo una relación teórica entre el porcentaje de materia orgánica en el suelo y las partes por millón por peso de los yerbicidas que se necesitan para lograr un 50 por ciento de reducción en el peso verde de la avena.


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