

## Characterization of humified compounds by extraction and fractionation on solid polyvinylpyrrolidone

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### ABSTRACT

New humification parameters are proposed for characterizing soils and organic fertilizers or amendments. They include an index, a degree and a rate of humification, all devised to assess the ratio between humified and non-humified materials. The separation of the two types of materials is essentially performed by extraction and fractionation on solid polyvinylpyrrolidone.

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### INTRODUCTION

Assessing quality criteria with respect to organic matter in soils, fertilizers, sludges or composts is a difficult task, and has led to controversial results. Some workers<sup>1</sup>, for instance, suggested the organic matter extraction of soil samples with a solution of 0.1 M sodium hydroxide plus 0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, but they did not apply this procedure to organic materials other than soil. Others<sup>2</sup> adopted parameters based on visible spectral properties (*e.g.*, on the degree of aromaticity), but found that the nominal molecular weight distribution of humic acids decreases with increasing degree of humification, which is the opposite of normally accepted trends. Also, the results obtained using the ratio between humic acids (HA) and fulvic acids (FA) are of uncertain interpretation because this ratio depends on many factors including even the geographical distribution of soils<sup>3</sup>. The  $E_4/E_6$  ratio, widely used as an index of humification<sup>4</sup>, depends on many factors, and the addition of a small amount of humic substances (*e.g.*, HA from leonardite) is sufficient to change the results completely. Humic substances have been defined as amorphous, polymeric, brown compounds; they do not belong to recognizable classes of organic compounds, such as polysaccharides, polypeptides or, altered lignins<sup>5</sup>. The procedure suggested by some workers<sup>1</sup> considers the alkaline extract (from soil or other organic materials) as the total humic extract, but it consists of both humic and non-humic substances. After precipitation of HA by acidification of the alkaline extract, the supernatant contains both FA and other classes of organic materials (*e.g.*, polysaccharides).

Polyamide columns have been used to retain the coloured fractions of FA<sup>6,7</sup>, whereas Amberlite XAD-8 has been preferred for adsorbing selectively humic substances from freshwater<sup>8,9</sup>. This last procedure has been recommended by the International Humic Substances Society (IHSS).

Polyvinylpyrrolidone (PVP), a cross-linked adsorbent for the chromatographic separation of aromatic acids, aldehydes and phenols<sup>10,11</sup>, has been used to separate phenols from different organic extracts<sup>12,13</sup>. Insoluble PVP has been found to allow strong adsorption and good recoveries of humic substances<sup>13</sup>.

The use of a selective extraction and fractionation on solid PVP has been suggested in order to distinguish non-humified from humified materials in soil, dung, compost and sludge extracts<sup>14</sup>. This paper reports further results obtained using this procedure.

## EXPERIMENTAL

### *Soil samples*

Soil samples were air-dried, crushed to pass a 2-mm sieve according to ISSS (Italian Society of Soil Science) methods<sup>15</sup> and stored in plastic bags.

### *Samples of organic materials*

Samples of organic fertilizers, amendments, composts, sewage sludges and swine slurries were dried at 40–50°C in a forced-air oven, crushed to pass a 0.5-mm sieve and stored in black plastic bags.

### *Preparation of the polyvinylpyrrolidone resin*

About 50 g of insoluble PVP resin (Aldrich) were placed into a 1-l glass cylinder. After washing twice with tap-water and twice with distilled water, discarding the fines each time, 0.005 *M* sulphuric acid was added and the suspension was stored at room temperature.

### *Recommended extraction and fractionation procedure*

Fig. 1 shows a scheme of the proposed extraction and fractionation procedure for organic extracts using insoluble PVP to separate non-humified materials in the so-called fulvic fractions. A standard procedure can be suggested as follows.

Place 10 g of soil sample or 2 g of organic material in a 150-ml centrifuge tube with 100 ml of 0.1 *M* sodium hydroxide plus 0.1 *M* Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> (NaOH + PP). Let nitrogen bubble through the solution for 2 min, then plug the tube immediately. Shake for 2 h at 160 oscillations per minute at room temperature, then centrifuge at 13000 *g* for 20 min. After centrifugation, filter through a 0.45- $\mu$ m Millipore filter using a vacuum pump. Transfer 25 ml of the extract (total extract, TE) into a centrifuge tube (usually 40 ml) and acidify to pH < 2 by adding a small volume (0.3–0.5 ml) of 50% sulphuric acid. Centrifuge at 5000 *g* for 20 minutes, collect and store the precipitate (apparent humic acid fraction, HA) and feed the supernatant solution onto a small column (normally a common 10-ml plastic siringe) packed with about 4–6 cm<sup>3</sup> of insoluble PVP previously equilibrated in 0.005 *M* sulphuric acid. Collect the eluate in a 50-ml volumetric flask, after discarding the first 2–3 ml, elute the column with about 20 ml of 0.005 *M* sulphuric acid, then dilute to volume with 0.005 *M* sulphuric acid and store for subsequent analyses (non-retained, or non-humified fraction, NH). Elute the retained fraction, generally brown (apparent fulvic fraction, FA), with 0.5 *M* sodium hydroxide solution, discarding the first 2–3 ml, and collect in the centrifuge tube containing the HA precipitate, which redissolves. Wash the col-

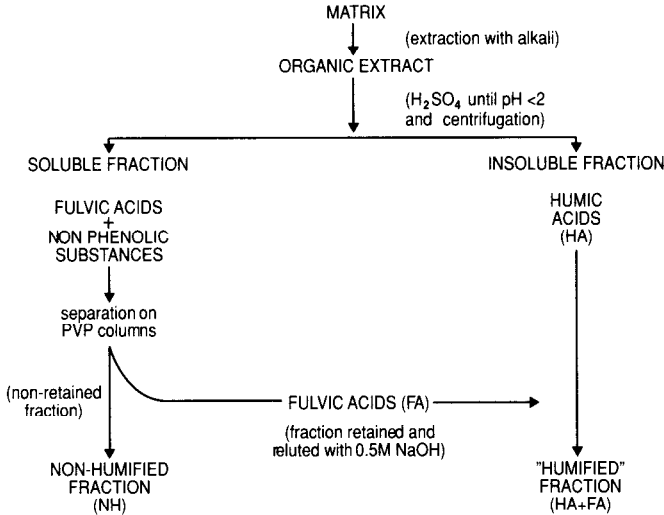


Fig. 1. Separation of humified (HA + FA) from non-humified materials (NH) by means of columns packed with insoluble PVP. Non-humified fractions are not retained on PVP; after washing with 0.005 M  $H_2SO_4$  the fulvic fraction is eluted with 0.05 M NaOH and added to humic acids.

umn with about 20 ml of 0.5 M sodium hydroxide solution, transfer the combined HA + FA fractions quantitatively into a 50-ml volumetric flask and dilute to volume with 0.5 M sodium hydroxide solution. Store the TE, NH and HA + FA fractions in a refrigerator at 4°C for subsequent analyses.

#### Determination of total organic carbon

Total organic carbon (TOC) was determined according to the dichromate acid oxidation method<sup>16</sup>. For liquid extracts (TE, NH and HA + FA), an aliquot (normally 1–10 ml, depending on the organic carbon concentration of the sample) was used for the determination according to the same procedure.

## RESULTS AND DISCUSSION

Some results achieved for selected samples of soils, organic amendments and fertilizers, composts, sewage sludges and swine slurries are given in Table I.

Three new parameters of humification are used in Table I, namely the humification index (HI)<sup>14</sup>:

$$HI = NH/(HA + FA)$$

*i.e.*, the ratio between non-humified (NH) and humified compounds (HA + FA); the degree of humification (DH)<sup>17</sup>:

$$DH(\%) = [(HA + FA)/TEC] \cdot 100$$

TABLE I

TOTAL ORGANIC CARBON AND INDEX (HI), DEGREE (DH) AND RATE OF HUMIFICATION (HR) OF SOME SAMPLES OF SOILS, AMENDMENTS, ORGANIC FERTILIZERS, COMPOSTS, SEWAGE SLUDGES AND SWINE SLURRIES

<i>Sample</i>	<i>Total organic carbon (%)</i>	<i>Humification index (HI)</i>	<i>Degree of humification (DH) (%)</i>	<i>Humification rate (HR) (%)</i>
Soil 1: protorendzina, A horizon	7.4	0.14	77.5	20.9
Soil 2: mollisol, A horizon	2.5	0.18	99.0	70.9
Soil 3: histosol	19.0	0.13	82.5	22.1
Soil 4: typic xeropsamments	1.5	0.42	63.9	19.0
Soil 5: fluventic xerochrept	1.9	0.47	67.9	17.1
Soil 6: podzol, A <sub>1</sub> horizon	7.5	0.28	68.0	21.8
Soil 7: podzol, B <sub>h</sub> horizon	2.7	0.17	71.0	35.4
Cow manure, well matured	18.5	0.32	76.0	8.0
Peat 1: Italy	31.1	0.30	76.9	19.3
Peat 2: Norway	48.1	0.29	77.6	22.1
Leonardite 1: U.S.A.	40.5	0.00	100.0	84.4
Leonardite 2: U.S.A.	41.3	0.05	94.9	82.7
Horn and hooves: Italy	35.6	2.87	25.9	4.2
Ground feather: Italy	47.0	34.00	2.9	0.2
Distillery washes: Italy	17.5	4.53	18.1	8.6
Leather meal: Italy	43.7	19.71	4.8	1.6
Compost: raw, from urban refuse	21.0	1.32	43.1	21.7
Compost: after stabilization	17.1	0.75	57.3	24.4
Sewage sludge: partially stabilized, from thickening bed	22.0	1.20	45.0	2.6
Sewage sludge: raw, from the outlet of anaerobic digester	26.0	2.13	31.0	2.8
Swine slurries: raw	49.5	1.20	35.7	13.9
Swine slurries: after 120 days of stabilization	30.4	0.80	48.7	12.5

*i.e.*, the percentage of humified compounds with respect to total extracted carbon (TEC); and the humification rate (HR)<sup>17</sup>:

$$HR(\%) = [(HA + FA)/TOC] \cdot 100$$

*i.e.*, the percentage of humified compounds with respect to TOC in the sample.

Table I shows that HI  $\approx$  0 (0–0.5) for humified materials (soils, organic amendments), and is much higher than 1 for non-humified materials (organic fertilizers, raw composts, sewage sludges and swine slurries). Fig. 2 shows the trend of HI during the organic matter stabilization processes in a compost from urban refuse. HI decreases continuously during humification processes, and reaches values lower than 1 at the

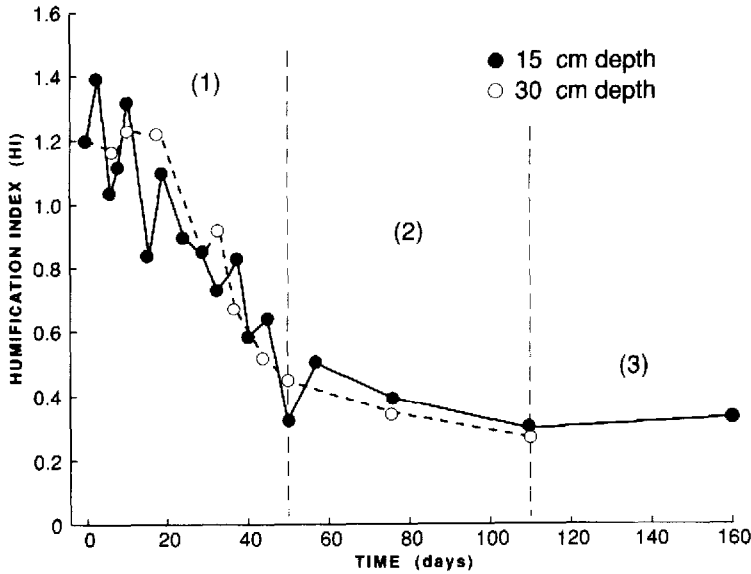


Fig. 2. Trend of the humification index (HI) during the (1) thermophilic, (2) mesophilic and (3) final phases of the organic matter stabilization process in a compost from urban refuse<sup>23</sup>.

end of the stabilization. In other studies HI has been used to characterize organic matter from animal manures after digestion by earthworms<sup>18</sup> and to evaluate the maturity of organic wastes<sup>19</sup> and organic matter stabilization in sewage sludges<sup>20</sup> and swine slurries<sup>21</sup>.

DH is higher than 60% for humified materials (soils, organic amendments), and close to 100 only for leonardites (fossil humic substances). Less humified samples (organic fertilizers, raw sewage sludges or swine slurries) show lower DH values. DH has also been used to monitor the evolution of organic matter from an organic fertil-

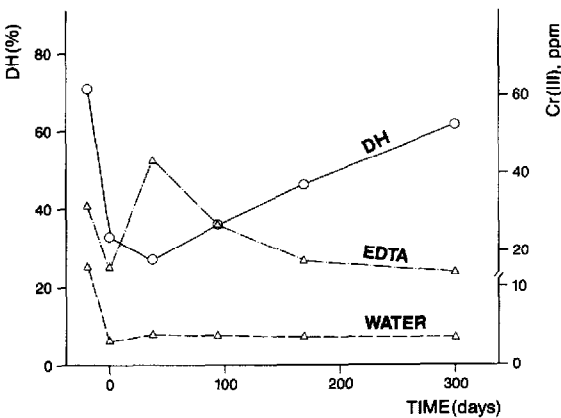


Fig. 3. Apparent degree of humification (DH) and soluble and EDTA-extractable chromium during humification of leather meal fertilizers in soil under aerobic conditions.

izer (leather meal)<sup>22</sup> even after application to the soil (Fig. 3) , in order to follow a possible chromium release during humification processes.

Strongly humified materials such as leonardites also show a high HR(>80%), whereas this parameter appears to be generally low for soils (except sample 2) and organic materials.

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