

Towards A More Safe Environment, Phytoremediation of Some Heavy Metals From Contaminated Soils In Egypt: Hydrophobic And Hydrophilic Fractions

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Abstract

Fulvic acid was extracted by HCl 0.1 N from El-Gabal El-Asfar area, Egypt (sandy soils under irrigation with sewage effluent for more than 80 years). The fractionation of fulvic acid (FA) into hydrophilic Organic Carbon). The XAD-8 resin was chosen as a stationary phase in the liquid chromatography separation. Determination of elementary composition, atomic ratios, functional groups (total acidity, carboxylic groups and phenolic-OH) and heavy metal content (Cd, Zn, Mn, Fe, Cu and Ni) of fulvic acid were carried out. The results showed the differences between sewage sludge-FA and soil-FA. While the first was observed to have an open and more aliphatic structure, the latter was more condensed and larger in particle sizes. The fractionation of the FA-hydrophobic fraction was taken place during the elution process from the resin column and different five fractions were obtained. The distribution of DOC and the studied heavy metals in the fractions (and subfractions as well) were recorded. Furthermore, the FTIR, UV-Visible light absorption and gel chromatography were studied. The obtained results showed distinct differences between the fractions. The aromaticity (hydrophobicity) and particle size were observed to be increased by the elution volume. Also, the responsible fraction about the chelation of each heavy metal was defined.

It was concluded that the DOC fractionation process has proven to be of a great value because it allowed to gain an insight into the diversity of organic compounds and their properties contribution.

Introduction

Most of chemicals are produced and used in efforts to improve human health, standard of living and safety through advancements in manufacturing, agriculture and agribusiness, medicine and to strengthen national defense.

However, the production, distribution, use, misuse, disposal, of these chemicals have polluted environments to levels that threaten the health of human, and indeed whole ecosystems balance or sustainability.

The choice of the most suitable treatment is based mainly on the assessment of the risk to the environment imposed by the presence of toxic substances in the soil. This risk assessment includes diagnosis (which is characterization of the polluted land at present time) and prognosis (which is a prediction of the evolution of the pollution with time as a result of a change in some soil parameter(s) such as pH, redox potential, . etc.

Traditional methods for the cleanup of pollution include the removal of the polluted materials and their disposal by land filling or incineration (so called dig, haul, bury, or burn methods (Roberts et al., 1988).

In spite of their high costs, they do not always ensure that contaminants are completely destroyed and the stabilization of many pollutants can not be guaranteed and their by-products will remain as a serious potential threat to ecosystems. Furthermore these methods have been known to generate even more toxic compounds.

Noteworthy, the chemicals from natural origins (animal, vegetable, or mineral) are otherwise stabilized through natural processes so, they do not accumulate to high levels. Therefore, we have to use technologies and practices that take advantage of natural systems (which are mostly Bio-systems) and processes to clean up pollution. Successful bioremediation strategies are those that are tailored to satisfy specific pollutant, site, public, regulatory, cost-and environmental effectiveness consideration.

Bio-remediation methods include :

- ❖ **Land farming** : which is the practice of spreading high concentrations of polluted material over a large uncontaminated area to encourage breakdown or stabilization of pollutants.
- ❖ **Biostimulation** : which involves stimulation of the numbers and activities of natural populations, usually bacteria or fungi, so they can better break down pollutants into harmless products.
- ❖ **Bio augmentation** : which is the practice of adding specialized microbes or their enzyme preparations to polluted matrices to accelerate transformation or stabilization of specific pollutants.
- ❖ **Composting** : which is based on an ancient technology for turning wastes into useable organic amendments. The reason for the persistence of a pollutant may be due to :
 - (1) Unbalanced and/or in appropriate levels of nutrients or aerations;
 - (2) Strong binding of the pollutant to the medium (adsorption) that prevents the availability of the pollutant for destruction or transformation,
 - (3) Inactivity of the native microbes caused by toxic concentrations. Accordingly, provision of appropriate nutrient and environmental conditions, which could

be given by composting, should allow natural pollutant clean up or stabilization to proceed. Additionally, some organic compounds formed during composting can bind some metals and prevent their translocation into sensitive ecosystems.

Phytoremediation is the use of certain plants withdrawal chemicals, compounds and heavy metals from the soil. Some plants can tolerate high levels of heavy metals and other toxic chemicals.

These plants are referred to as hyper accumulators. The best plants used in phytoremediation are those plants that have crop characteristics that are tall, high yielding, fast growing, and easy to harvest. There are three major factors required by plants in order to be used as hyper accumulators (1) plants must be able to tolerate high levels of the element or compounds in root and shoot cells, (2) the plant must have the ability to translocate an element from roots to shoots at high rates, (3) the element or compound must be in solution and at a high enough concentration level to be taking up by the plant.

Phytoremediation which is a particularly appealing technology because it employs a more familiar system, plants rather than invisible microbes. The flexibility of this method is afforded by two evolutionary adaptations :

- (1) certain plants have evolved the capacity to take up and accumulate selected metals in their shoots and roots at levels that are toxic to ordinary plants; (Ebbs and Kochian, 1997).
- (2) Plants have evolved interactions and association with organisms that can cause accelerated breakdown or transformation of certain pollutants in the plant root zones to products that no longer pose environmental hazards (Becker, 2000).

Phytoremediation has several appealing attributes: a) it is an in situ technology, b) it is applicable to the treatment of polluted soils as well as polluted water systems, c) it could be used to clean up a broad range of contaminants including petroleum hydrocarbon, as well as inorganic pollutants, d) it is no longer limited to the clean up of surface contamination, and now, trees with deep rooting systems and high evapotranspiration rates are being used to intercept and remove pollutants from subsurface environments.

Not only do these plants uptake the unwanted materials, but such plants also limit the movement of materials within the soil and in some cases those materials can be extracted from the plant and reused. Other plants can convert the toxic material into a non-toxic or non-bioavailable form (Brown et al.,1994). Since the environmental movement of the 1960s and 1970s large efforts have been conducted to reclaim contaminated lands. Phytoremediation has been in use for several years. Researchers have learned, studied, and manipulated this concept to clean up areas such as dilapidated mine sites, dump sites, soils with high levels of pesticide and herbicide

residue. Phytoremediation has been used in soils surrounding the United States government's explosive storage areas, scientists have been able to start reclaiming the soil and water surrounding Chernobyl Russia by sunflowers. The sunflowers can reduce the amount of Uranium concentration in the water up to 95%. The lead and zinc that was once of hazard is now being recycled from the harvested plants grown in a site containing buried car batteries in New Jersey. In meantime, many worker have been investigating the phytoremediation in Egypt (Ali, 1996, Kamal, 1999, Al-Salama, 2002).

The intensive cultivation depletes some plant nutrients in Egyptian soils. Also, the majority of the newly reclaimed soils for agricultural expansion are sand in texture and poor in plant nutrients content.

It is well known that the sewage sludge amendments, that are incorporated or surface applied, result in improved soil physico-chemical conditions. Beside the contribution of organic matter, sewage sludge provides significant contributions of N, P and K as well as micronutrients. However, undesirable constituents potentially associated with sewage sludge application include elevated levels of heavy metal which considered as toxic elements.

Also, Persicani (1995) stated that the increasing waste production by human activities increases the need for finding adequate terrestrial sites where such materials can be placed.

Therefore, land application of effluents has been discharged for along time ago in some sandy soils in El-Gabal Al-Asfar area. Although the application of sludge on land may be considered a recycling process for the essential nutrients, El-Nennah and El-Kobbia (1983) concluded that attention should be focused on the accumulation of heavy metals in soil and plant. Also, Eid (1991) found that the recorded increases for the heavy metals content in this area by time (9,60 and 100 times for total content and 15, 60 and 50 times for available content of Ni, Cu and Zn respectively) in 30 years. Therefore, this area could be considered as contaminated soils after 90 years discharging of sewage sludge. In view of the existent literature of metal complexation by components of natural soil organic matter, it is reasonable to suggest that the soluble fraction (Fulvic acid) of sludge amended soil will play an important role in determination of the enhancement in trace metals solubility.

Therefore, the objective of this study was to study the fractionation of soluble organic matter to provide a basis for more accurate prediction of heavy metal content in soil solution and then the availability of them. Also, to examine phytoremediation method by using Soybeanay Crop.

Materials and methods

Surface soil samples (0-30 cm) were collected from El Gabal El-Asfar area (old farm) for physical and chemical analysis. The soluble fraction of organic matter

was extracted and fractionated. Pot experiment was carried out to examine the phytoremediation method by using soy bean crop.

Different soil samples were used in pot experiment :

- ❖ 100% El-Gabal El Asfar Soil
- ❖ 50% El-Gabal El-Asfar Soil + 50% pure sand
- ❖ 25% El Gabal El-Asfar Soil + 75% pure and

The plants were cut when it was 30 cm hight.

Mechanical analysis, organic matter content, pH values, total and available Fe, Mn, Zn and Cu in soil were determined. The methods used are explained by Abd-El Naby (2002). Also the elements content in roots and shoots of plants. Organic matter and dissolved organic carbon, fulvic acid extraction and fractionation, infrared analysis and heavy metal content in the fractions were carried out and explained by Taha and Stevenson (1991).

Results and discussion

The physical and chemical properties of the studied soil are presented in table (1). The values in this table show that it is a sandy soil with high content of organic matter and also a high pH. Therefore, the heavy metal ions could be expected to be insoluble in such high pH (8.05). The high content of organic matter in sandy soil could be the limiting factor of solubility and availability of heavy metal content. Thus, the fractionation of soluble organic matter presented as Dissolved Organic Carbon DOC into hydrophobic and hydrophilic fraction should be carried out.

Table (1) Some physical and chemical properties of studied soil:

Depth	0-30	Soluble ions	Meg/liter
Texture :		Ca ⁺⁺	3.5
Coarse sand %	65.43	Mg ⁺⁺	2.4
Fine sand %	24.30	Na ⁺	5.2
Silt %	2.61	K ⁺	1.2
Clay %	7.66	So ₄ ⁻	2.0
pH ⁺	8.05	HCo ₃ ⁻	6.3
EC ⁺ mmhos/cm	2.64	Cl ⁻	4.0
O.M %	6.17		

* In the extract of the saturated soil paste.

Fractionation of humic substances by adsorptive interactions has been the most successful method for fractionation as well as concentration and isolation of aquatic humic substances. Humic substances readily interact with various adsorptive surfaces without the requirement of crossing the interface as is necessary with solvent partitioning (Leenheer 1985). Normal-phase chromatography with aqueous mobile phases has been found to fractionate aquatic humic substances according to the nature of their polar functional group content. In recent years, it has been found that high recoveries of organic compounds are possible with non-ionic macroporous sorbents such as the Amerlite XAD resin series which have large surface area. The hydrophobic effect is the principal driving force for sorption on these resins. Particularly, XAD-8 is more efficiently eluted than the other types of XAD when fulvic acid is solute of interest (Aiken, 1985).

In table (2) the results of elementary composition, atomic ratios and oxygen-containing functional groups are presented. The sewage sludge-FA contains a large percentage of ash and also differs from those of soil organic matter-FA. The high content of O₂ and hydrogen shows that a large portion of sludge-FA is in aliphatic structure and has a high degree of oxidation. The ratio of H/C is higher than those reported for soil-FA. However, it could not be taken as estimate of the aromatic C content of the studied FA as indicated by Gauthier et al., (1987) since the molecular groups COOH and HC=CH both have the same H/C ratio. So, it is not surprising that the H/C ratio was found to be less strongly correlated to aromatic carbon (Traina et al., 1990). Moreover, the results of the functional groups confirm that sewage sludge-FA has an open structure with a high degree of oxidation. Noteworthy, a such component with all of these active sites should play a very important role in the mobility, as well as the fixation of heavy metals depending on the type of fraction: hydrophobic or hydrophilic. Also, the same observation could be sustained by the high value for E₄/E₆ ratio. Conversely, a high E₄/E₆ ratio reflects a low degree of aromatic condensation and infers the presence of relatively large proportions of aliphatic structure. Chen et al., (1977) concluded that the E₄/E₆ ratio was governed by particle size and/or molecular weight and related to free radical concentration, contents of COOH and total acidity.

Table 3: Dissolved Organic Carbon (DOC) and heavy metal distribution (ppm).

	DOC	Cd	Zn	Mn	Fe	Cu	Ni
Total	437.3	19	9811	66	8823	5364	319
Hydrophilic	318.6	14	6756	29	7329	2159	178
	72.9%	73.7%	68.9%	43.9%	83.1%	40.2%	55.8%
Hydrophobic	118.7	5	3055	37	1494	3205	141
	27.1%	26.3%	31.1%	56.1%	16.9%	59.8%	44.2%

The results of DOC and heavy metals distribution within the hydrophobic and the hydrophilic fractions are presented in table 3. The majority of organic carbon is existing in the hydrophilic fraction. Some of determined heavy metals were shown to be favored by the hydrophilic (Fe, Cd and Zn) while others were attracted to the hydrophobic fraction (Cu, Mn and Ni). However, no natural cutoff exists between these two fractions due to the equilibrium in soil environments. Elliott et al., (1990) indicated that a small portion of the sludge Cd was in a readily exchangeable form, whereas the majority is organically bounded. Also, Zn migration through soils was found to depend more on the organic C concentration of leachates than on soil characteristics (Fresques et al., 1990). It is worthy to mention that the hydrophobic properties make these compounds resist the biodegradation and have long residence times in soils (Schulten and Schintzer, 1990).

When the hydrophobic fraction was adsorbed on the resin (XAD-8), a narrow band at the top was observed. At low pH, the acids are protonated and adsorbed on the resin while at high pH (when it was eluted by NaOH) the acids are ionized and desorption is favored. Because of the differences in the degree of association among the hydrophobic fraction, subfractions were obtained at different elution volumes. The results of DOC and studied heavy metals distribution in the hydrophobic subfraction are shown in Fig. 1.

Obviously, each patch contains different amount of DOC and a certain heavy metal. The first patch of the next fraction contains DOC higher than the last patch of the previous one which elucidates the discrete difference between fractions. By monitoring the pH during the elution process from XAD-8 resin, the different five fractions were collected at different pH values (below 3, 5, 8, 10 and higher than 11). Thereby, different amounts and types of acidic functional groups in separated fractions could be confirmed. Therefore, it could be anticipated that a such fractionation process could be used in the study of the affinity constant for metal ions by different humic fractions.

Infrared spectra (FTIR) of different fractions are reproduced in Fig. 2. The interpretation of the spectra are based on Bellamy (1975), Stevenson (1982), and MacCarthy and Rice (1985). The main absorption bands assignments are given in table 4.

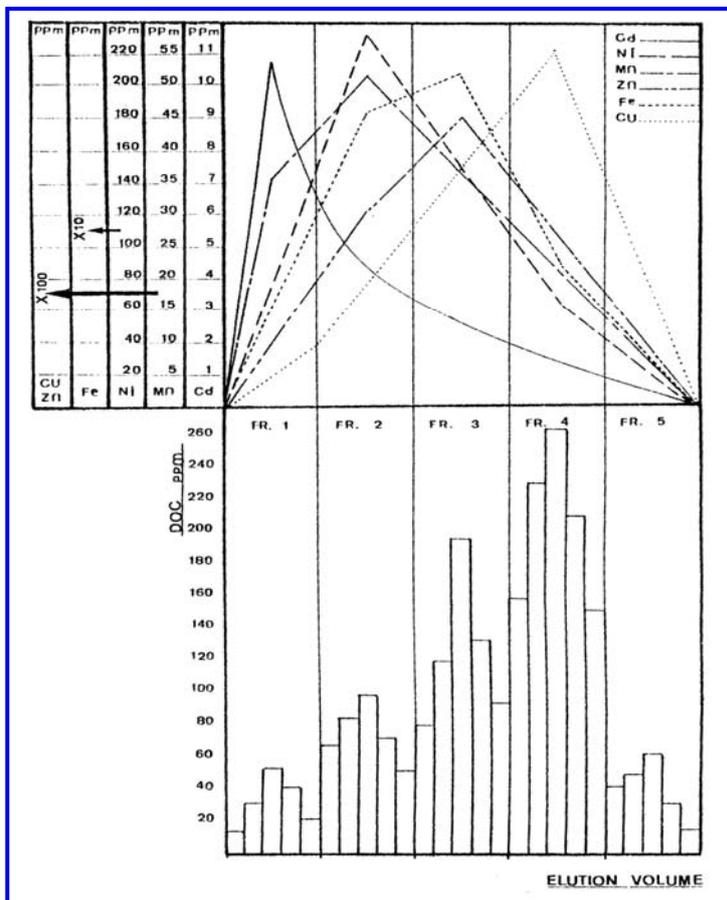


Fig. 1 : DOC and heavy metals distribution in the hydrophobic fractions and subfractions.

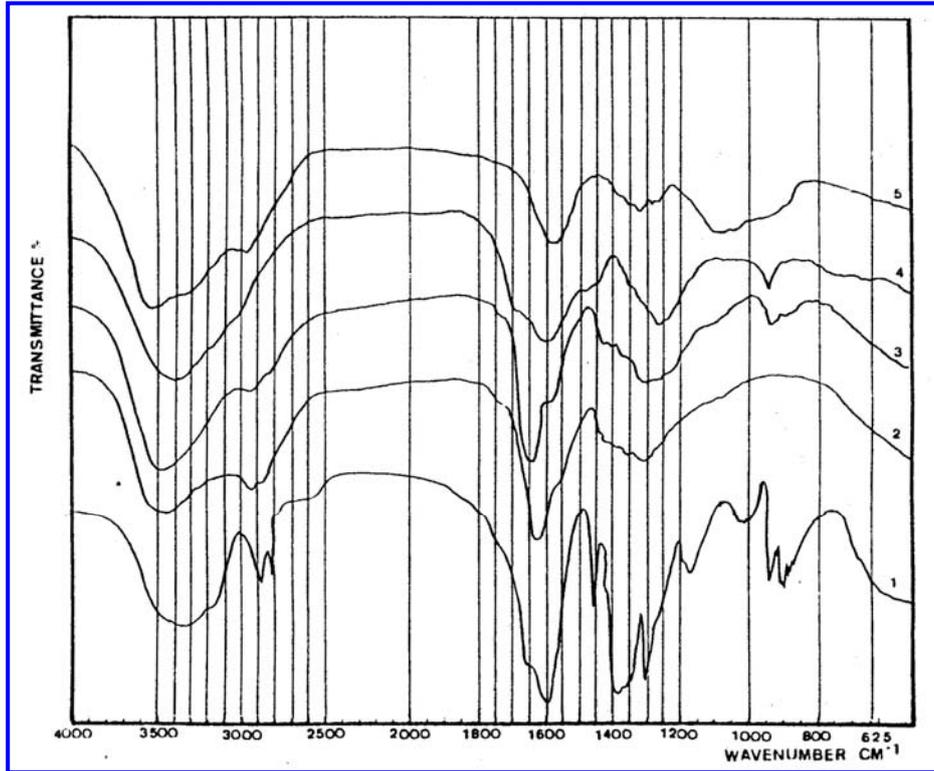


Fig. 2: FTIR spectra of different hydrophobic fractins separated by XAD-8 resin

Table 4: The main absorption bands assignments.

Wave number Cm^{-1}	Interpretation
2930,2850	Aliphatic C-H stretch
1730 to 1715	C=O of COOH, C=O of ketonic carbonyl
1665 - 1630	C=O stretching of amid egroups (amide I) quinone, C=O stretching of H-bonded conjugated ketons
1590 - 1517	COO^- symmetric stretching, N-H deformation, and C=N stretching (amide II band)
1510	C=C of aromatic rings
1460	Aliphatic C-H
1420-1390	Aromatic ring stretch, COO^- antisymmetric stretching
1400-1300	Aliphatic C-H groups
1290-1200	C-O stretch of OH-deformation of COOH
1170-950	C-O stretch of polysaccharides
1160	S=O in sulfone groups
880	Hydrogen bonded OH- deformation in carboxhyl groups

The spectras of the different fractions obtained expose broad absorption peaks. This may be due to the extensive overlapping of individual absorption bands. As a general observation, all the spectra show the principal features of the spectra of soil humic substances reported by Stevenson and Goh (1971); Distinctly, differences between the fractions could be observed. The changes in the center of the shoulder between 3500 and 3000 cm^{-1} show types of OH groups and N-H stretching in the fractions. The peaks between 2900 and 2800 cm^{-1} , at 1450 and at 1300 cm^{-1} were eliminated which explain a decrease in the aliphatic structure by elution volume. Also, the shift in peaks at 1720, 1600 and 1400 cm^{-1} observes the different substitutions in the molecules near by the carboxylic groups and/or carboxylic groups deformation. Clearly, the IR spectrum of the first fraction exhibites pronounced peaks between 950 and 1170 cm^{-1} which could be attributed to C=O stretch of polysaccharides and/or S=O in sulfone groups.

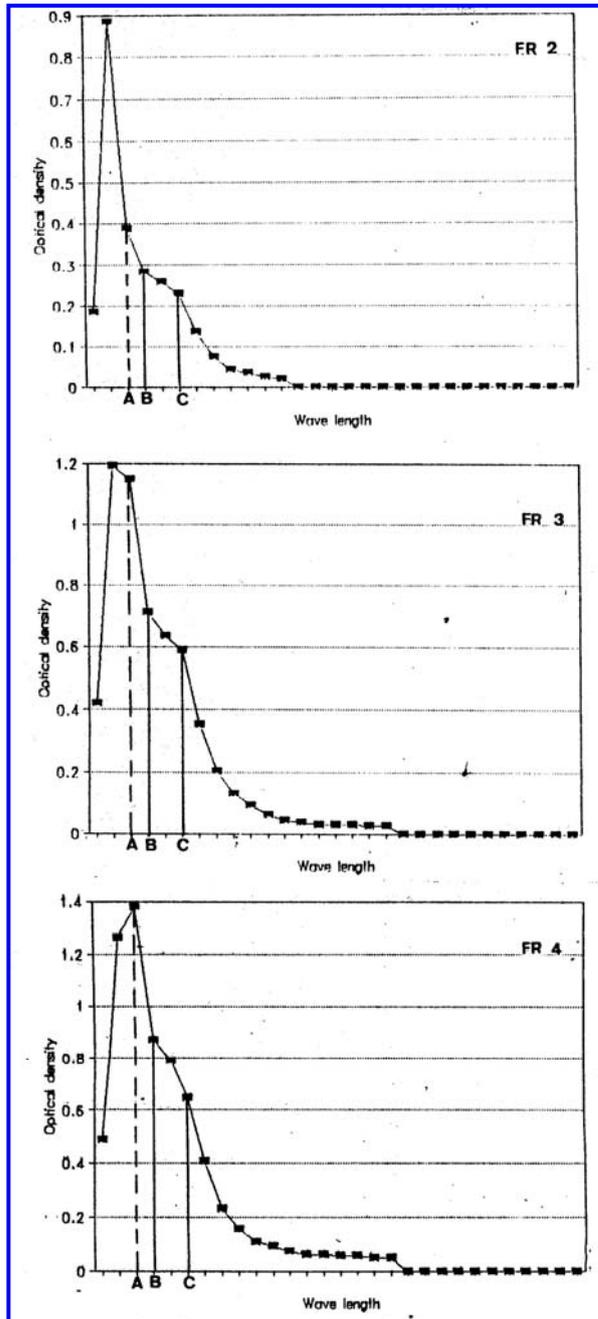


Fig. 3: The Ultraviolet (UV) - Visible light absorption by different hydrophobic fractions (2, 3 and 4) (A, B and C represent UV absorbance at 210, 240 and 280 respectively).

The differences between fractions were supported by studying the UV-visible light absorption. The Ultraviolet (UV) Visible spectra of humic substances are essentially featureless. The absorbance decreased with increasing wavelength. However, the spectra of different fractions had a pronounced shoulder centered at about 240 to 280 nm (B and C on the spectra) as shown in Fig. 3. This shoulder could be attributed to a quinonoid moiety. The enhancement of this shoulder was found to be due to a decrease in hydrogen ion concentration and/or intramolecular hydrogen bonds deformation (Ghosh and Schnitzer, 1979). Also, it was observed that the absorbance at 210 nm is due to the intense absorbance by the π () electrons in aromatic ring (Baes and Bloom, 1990). It was suggested that determination of UV absorptivity can be used to provide a quantitative estimate of the aromatic C content (Trania et al., 1990). Clearly, a measure of the aromaticity of a particular organic fraction would be of value in estimating its effect on the fate of pollutant in natural environments.

Thus, it could be detected that the different fractions represent different degrees of aromaticity and then reactivity in soils.

The rationale for the fractionation, provided above, was further substantiated by gel chromatography. During the separation by gel filtration, small particles move with the eluant both within and without the gel particles, while large particles move outside the gel particles only. Thus, the large molecules are eluted first followed in order by the smaller. Thereby, it could be deduced that the fractions differ in particle size distribution with greater portion of larger particles is presented in fraction No. 4.

Obviously, the procedure of DOC fractionation provides a rapid and convenient method for separation of different hydrophobic and hydrophilic fraction according to the polarity, DOC distribution, heavy metal content (quality and quantity), aromaticity and particle sizes. Thus, it leads to relatively homogeneous fractions and it has proven to be of great value because it allowed to gain insight into the diversity of organic compounds and their properties contribution. Also, this process could be used to identify the responsible fraction for a certain heavy metal interactions since the lack in selectivity of heavy metal extractants dictates caution when interpreting the normal extraction schemes.

The adsorption chromatography of humic substances is still in its developmental stages. However, the rapid advance being made in the field of liquid chromatography undoubtedly will have applications to the fractionation of humic substances.

Pot Experiment :

Soybean (CV Williams 82) was grown in each of the three soils (100, 50, 25% soil of El Gabal El Asfar). The first results showed that the uptake of heavy metals increased as the amount of sludge (% of El-Gabal El Asfar soil) increased. However, more attention has to be paid to metal-hyperaccumulating mechanisms which involve extracellular and intracellular metal chelation, precipitation and translocation in the vascular system. These mechanisms are still poorly understood. Also, information about

metal-binding proteins, metallo-enzymes and various storage, carrier and channel / transport proteins still unknown.

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