Chapter 1

REMEDIATION CHEMISTRY OF HUMIC SUBSTANCES: THEORY AND IMPLICATIONS FOR TECHNOLOGY

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Abstract

An overview is given of the interactions encountered between humic substances (HS), ecotoxicants, and living organisms in the context of environmental remediation. The most important interactions identified include: binding interactions affecting chemical speciation and bioavailability of contaminants; interfacial interactions altering physical speciation or interphase partitioning of ecotoxicants; abiotic-biotic redox interactions that influence metabolic pathways coupled to pollutants; and finally direct and indirect interactions coupled to various physiological functions of living organisms. Because humics are polyfunctional, they can operate as binding agents and detoxicants, sorbents and flushing agents, redox mediators of abiotic and biotic reactions, nutrient carriers, bioadaptogens, and growth-stimulators. It is shown that these functions possess significant utility in the remediation of contaminated environments and as such humic-based reactions pertinent to permeable reactive barriers, *in situ* flushing, bioremediation, and phytoremediation are examined in detail. Finally, this chapter introduces the novel concept of “designer humics” which are a special class of customized humics of the reduced structural heterogeneity and of the controlled size. They are developed and deployed to carry out one or more of the above *in situ* functions in an optimum manner and for the purpose of enhancing the efficacy of one or more remediation technologies. Designer humics possess specified reactive properties obtained by chemical modification and cross-linking of the humic backbone. This new class of reactive agents portend new opportunities for achieving enhanced remediation and for quantifying remediation performance. The latter is described in the context of the passive flux meter technology developed for direct measuring fluxes of contaminants and biomass.

1. Introduction

Effective remediation of polluted environments is one of the crucial issues on Agenda 21, which lists priorities for achieving sustainable development [1]. Eastern and Western

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countries alike are currently facing environmental reclamation costs that are increasing exponentially. In the U.S. and Europe, remediation costs generally exceed the net economic value of the land, and often threaten responsible companies with bankruptcy. Given this perspective it is not surprising that scientists and engineers on both sides of the Atlantic have aggressively tried to develop novel technologies to meet regulatory standards at a fraction of the costs associated with traditional approaches (incineration, pump-and-treat, etc.) [2-7].

New remediation technologies are often discovered in process of overcoming limitations of current technologies, and *in situ* remediation is one novel class of technologies that shows considerable promise from both technical and economic perspectives [8-10]. *In situ* remediation relies upon natural and enhanced processes that govern the fate and transport of chemicals released in environment. To a large extent, the reliance on natural processes is predicated on a desire to control costs [11]. Thus, *in situ* technologies that deploy natural attenuating agents such as humic substances (HS) may be even more cost effective.

HS are ubiquitous in the environment and comprise the most abundant pool of non-living organic matter [12]. Their peculiar feature is polyfunctionality, which enables them to interact with both metal ions and organic chemicals. The palette of potential interactions includes ion exchange, complexation, redox transformations, hydrophobic bonding, etc. As a result, numerous studies have shown humics capable of altering both the chemical and the physical speciation of the ecotoxicants (ET) and in turn affecting their bioavailability and toxicity [13]. Hence, HS hold great promise functioning as amendments to mitigate the adverse impacts of ET and as active agents in remediation.

The goal of this chapter is to elucidate emerging concepts of HS-based remediation technologies. Thus, the objectives are: (1) to categorize the interactions encountered between humics, ecotoxicants and living organisms in a polluted environment in the context of remediation chemsitry; (2) to assess the scope of current remedial applications of humics, and (3) to define promising directions of technological developments for remedial implementation of humics.

2. Basic definitions and main features of humic substances

2.1. GENESIS, SOURCES, AND RESERVES OF HUMIC SUBSTANCES IN THE ENVIRONMENT

Humification is the chemical-microbiological process of transforming debris from living organisms into a general class of refractory organic compounds otherwise known as humic substances. It is the second largest process after photosynthesis and involves 20 Gton C/a [12]. Humic substances account for 50 to 80% of the organic carbon of soil, natural water, and bottom sediments [14-16].

Humic materials are typically derived on an industrial scale from peat, sapropel, and coal. *Peat* is a heterogeneous mixture of more or less decomposed plant material (humus) that accumulated in a water-saturated environment in the absence of oxygen [17]. *Coalification* of plant debris preserved in peat mires leads to the formation of *humic coals*. Terms like *peat*, *lignite*, *subbituminous*, *bituminous* and *anthracite* indicate different stages of the coalification process, and they also denote the rank of various coals. The term "*brown coal*" is often used for lignite and subbituminous coals, while "*hard coal*" indicates coals of higher rank. The net result of coalification is an extension of the humification process to include a continuous enrichment of fixed carbon with increasing rank. The relevant increments of carbon content, or % of the total mass, range from: 10-30 (peat), 30-40 (lignites), 40-65 (subbituminous), 65-80 (bituminous), and over 80 (anthracite) [18]. *Sapropel* is an unconsolidated sedimentary deposit rich in *bituminous* substances [19]. It is distinguished from peat in being rich in fatty and waxy substances and poor in cellulosic material. When consolidated into rock, sapropel becomes oil shale, bituminous shale, or *sapropelic* (boghead) *coal*.

The richest source of HS is *leonardite*, a soft brown coal-like deposit usually found in conjunction with deposits of lignite. Leonardite is the most widely used raw material for production of commercial humic preparations [20] followed by other low-rank coals, peat, and sapropel. Table 1 shows the reserves of inexpensive humics-rich materials are immense; however, these reserves are not currently being tapped for environmental remediation.

Table 1. Reserves of humic materials of industrial value.

Source	Amount, Gton C	Ref.
Lignite and Subbituminous coal (Total/Recovered)	1120/512	[21]
Anthracite and Bituminous coal (Total/Recovered)	3880/571	[21]
Peat	400-500	[22]
Sapropel	800	[23]

2.2. CLASSIFICATION, STRUCTURE AND REACTIVITY OF HUMIC SUBSTANCES

Being the products of stochastic synthesis, HS have an elemental composition that is non-stoichiometric, and structure which is irregular and heterogeneous [24]. Aiken et al. [14] defined HS as "a general category of naturally occurring, biogenic, heterogeneous organic substances generally characterized as yellow to black in colour, of high molecular weight, and refractory". MacCarthy and Rice [25] hypothesized that the structural heterogeneity of humics may explain their resistance to biodegradation as longevity of HS in soils is typically on the order of thousands of years. The recalcitrant nature of humics is of practical relevance particularly when the objective is to develop soil/aquifer remediation technologies predicated on a reactive matrix that is not consumed by microorganisms during remediation.

The best illustration of the stochastic nature of HS is provided by the structural model of Kleinhempel (1970) [26] depicted in Figure 1. Clearly, as shown in this figure, a single structural formula cannot be ascribed to any humic sample; consequently, current definitions and classifications of HS are based on isolation procedures rather than on specific molecular features. Thus, the most commonly applied classification is based on humic constituent solubilitiy in dilute acids and bases [27]: *humic acids* (*HA*) represent the fraction that is insoluble at pH<2, *fulvic acids* (*FA*) constitute the fraction soluble under all pH conditions, and *humin* is the fraction insoluble under all pH conditions. Alkali extraction is the most common industrial technique of preparing humics from brown coal or peat and the resultant salts of humic acids are called *humates*. Humates of sodium, potassium, and ammonium comprise the major fraction of the commercially available humic products. The term “humates” is often used to designate any commercially available humic-based product; however, in this chapter, the term is used only to designate the alkali/alkali-earth metals or ammonium salts of HA.

Despite its stochastic nature, HS from different sources share common elements of structural organization. The average humic macromolecule consists of a hydrophobic aromatic core that is highly substituted with functional groups (mostly carboxyl and hydroxyl), and with side aliphatic chains. The core is ensconced in a periphery of hydrolysable carbohydrate-protein fragments [15, 28]. The mass fraction of peripheral fragments decreases with humification; hence, the contribution of labile fragments is greatest among humics derived from composts followed by peat, soil, and finally coal. Coal-derived humics are enriched in condensed aromatic structures and depleted in aliphatic carbohydrate moieties; thus, these humics are much more hydrophobic and less biodegradable than their peat-based counterparts.

The structural complexity inherent in HS creates opportunities for a broad range of chemical interactions as indicated in Figure 2. Humics can be oxidized by strong oxidants; act as reducing agents; take part in protolytic, ion exchange, and complexation reactions; participate in donor-acceptor interactions; engage in hydrogen bonding; and take part in van-der-Waals interactions [29 and citations in it]. Hence, HS can interact practically with all chemicals released in the environment. More pertinent, however, is that humics interact with all classes of ecotoxicants including: heavy metals, petroleum and chlorinated hydrocarbons, pesticides, nitroaromatic explosives, azo dyes, actinides, etc. as shown in Figure 3. Indeed, humics are known to form stable complexes with heavy metals [30-34] and adducts with hydrophobic organic compounds [35-38]; produce charge-transfer complexes [39, 40]; act as electron shuttles [41, 42] and mediate redox reactions of transition metals [43], of chlorinated and nitrated hydrocarbons [44, 45]; adsorb onto mineral surfaces [46, 47]; and influence the interphase distribution of the contaminants [48, 49]. Finally, humics can strengthen the resistance of living organisms against non-specific stress factors [50, 51].

This unique constellation of reactive features strongly suggests HS have the potential to address a broad spectrum of needs within the focus area of environmental remediation [53]. This theoretical statement is confirmed by multiple examples of actual applications in remediation [54-58]. However, to ensure optimum and systematic application, an expanded knowledge base is needed concerning interactions between humics, ecotoxicants, and living organisms.

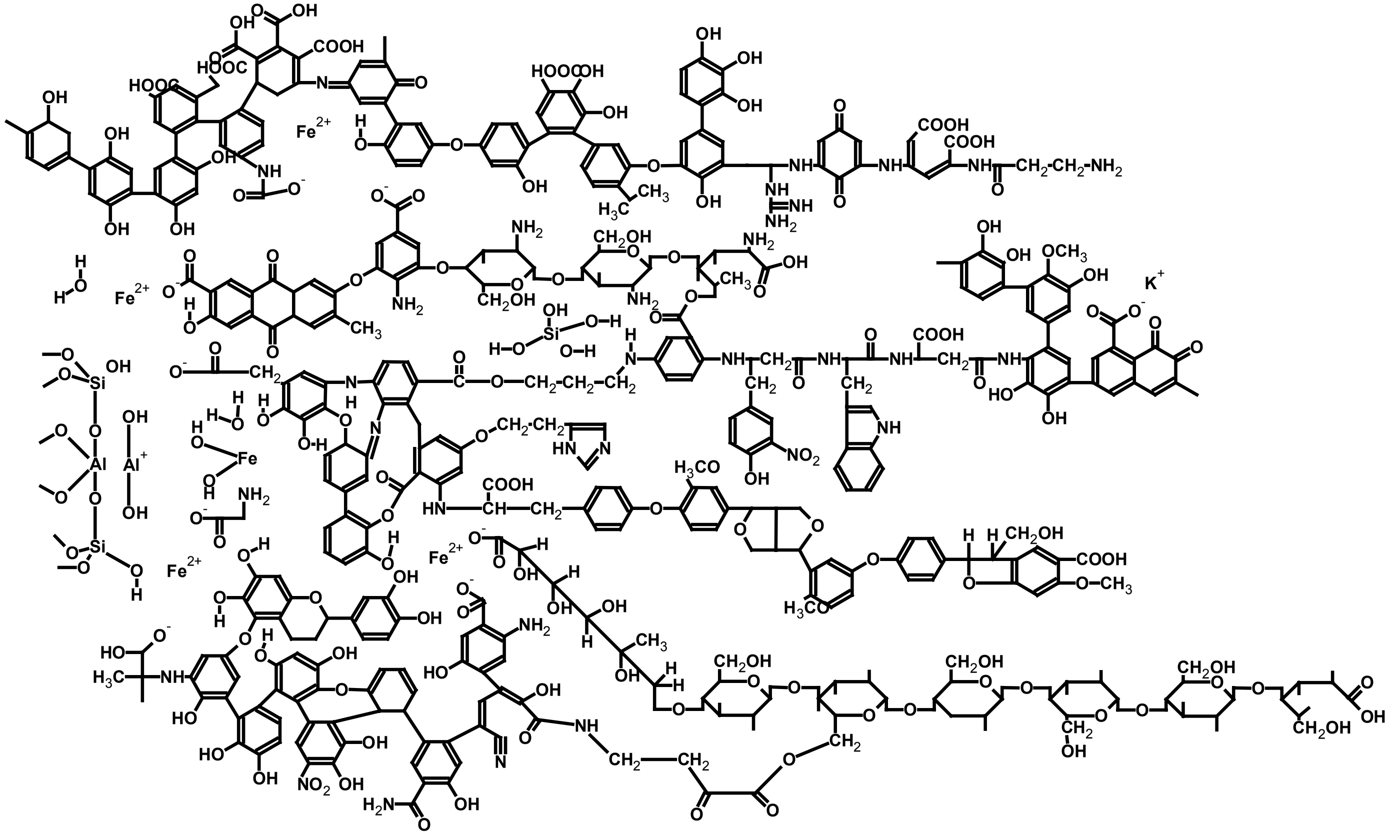


Figure 1. Hypothetical structural fragment of soil humic substances by Kleinhempel [26] that illustrates stochastic nature of humics.

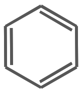
Structural moiety	Type of related interaction
- COOH	ion-exchange, complexation
- OH	complexation, hydrogen bonding
>C=O	reduction-oxidation
	donor-acceptor interaction (charge transfer complexes)
- CH_n	hydrophobic interaction

Figure 2. Diversity of structural moieties inherent in HS provides a broad range of chemical interactions they are able of. Humics can take part in protolytic, ion exchange, and complexation reactions; participate in donor-acceptor interactions; engage in hydrogen bonding; and take part in van-der-Waals interactions

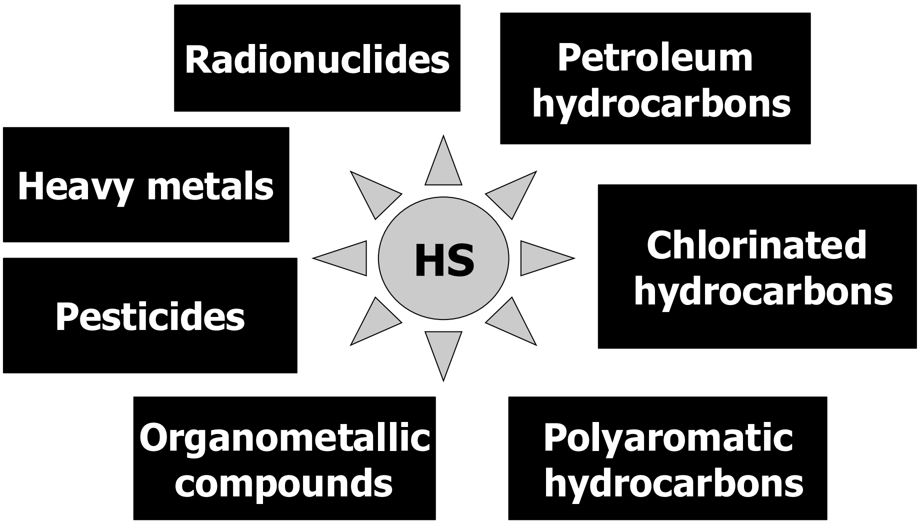


Figure 3. As a result of the diverse reactivity of HS, they can interact with all classes of ecotoxicants (ET) in the polluted environment. Humics are known to form stable complexes with heavy metals and radionuclides; to produce adducts and charge transfer complexes with hydrophobic organic compounds; to mediate redox reactions of transition metals, chlorinated and nitrated hydrocarbons.

3. Remedial properties of HS in polluted environments

Multiple interactions between HS, ecotoxicants, and living organisms may be organized to include:

- binding interactions that impact chemical speciation and bioavailability of ecotoxicants;
- sorptive interactions affecting physical speciation or interphase partitioning of ecotoxicants;
- abiotic-biotic redox interactions that impact metabolic pathways coupled to ecotoxicants; and,
- direct and indirect interactions with various physiological functions of living organisms.

To assess the extent to which the above interactions translate into properties pertinent to environmental remediation, each will be considered in the context of the needs and the limitations existing among *in situ* remediation technologies.

3.1. BASIC CONCEPTS AND NEEDS OF *IN SITU* REMEDIATION TECHNOLOGIES

In situ remediation relies upon natural or enhanced processes and does not imply the removal of contaminated soil or the extraction of polluted groundwater [59]. The various *in situ* remediation technologies can be organized to include [60]:

physical treatment: air sparging; directional wells; electrokinetics; fracturing (blast-enhanced, hydraulic, pneumatic); thermal enhancements; vacuum extraction, etc.;

chemical treatment: flushing; permeable reactive barrier (PRB) and treatment walls; immobilization/solidification; etc.; and

biological treatment: intrinsic bioremediation, enhanced bioremediation, phytoremediation, etc.

Most remedial technologies can be applied as a combination of physical-chemical, chemical-biological, or physical-chemical-biological treatments. The details of the above technologies will not be reviewed here but can be found elsewhere [59, 60], while corresponding reviews and case studies are available from relevant web-sites [4-7]. The most promising opportunities for the application of humics-based products and for the development of new humics-based remediation technologies are those predicated on strategies of *in situ* chemical and biological treatment. Examples of these technologies are given in Figure 4 and briefly described in the following paragraphs.

PRBs are replaceable or permanent units installed across the flow path of a contaminant plume. The plume is allowed to migrate passively through the PRB and in the process contaminants are precipitated, sorbed, or degraded [61, 62]. PRBs are filled with different reactive materials such as metals or metal-based catalysts for degrading volatile organics, chelators or ion exchangers for immobilizing metal ions, nutrients and oxygen for microorganisms to enhance bioremediation, or other agents. The reactions that take place in barriers are dependent upon parameters such as pH, oxidation/reduction potential, concentrations, and kinetics. Reactive materials used must demonstrate sufficiently rapid kinetics to remove target contaminants from ground water under natural gradient conditions. In addition, these reactive materials must be inexpensive and functional over an extended time horizon. Finally, pertinent chemical reactions must not produce and release toxic by-products. To date, a limited number of reactive materials satisfy these restrictions including zero valent iron (ZVI) – the most frequently utilized medium, zeolites, peat, lime and ferric oxyhydroxide [62, 63]. Humic-based materials show considerable promise as refractory and inexpensive reactive PRB components. This is particularly true wherever remediation involves a complex array of contaminants, and the reactive material must treat both soluble heavy metals and hydrophobic organics [54]. To evaluate the potential for using HS in PRBs, it is important to understand the sorptive and the redox properties of humics, and both are considered later in this chapter. For a case study on the application of humics in a sorptive PRB see Balcke et al. [64].

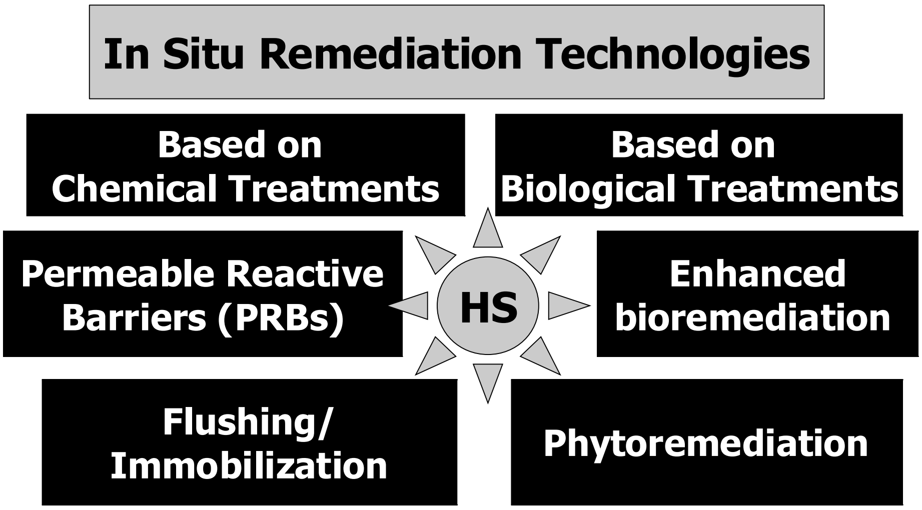


Figure 4. The technologies predicated on strategies of *in situ* chemical and biological treatment can be considered as target remediation technologies for application of HS-based products. The diagram shows the most promising examples of those.

In situ flushing involves the injection or infiltration of an aqueous solution into a zone of contaminated soil or aquifer [60]. The injected fluid functions to increase the mobility and/or solubility of immobilized contaminants. Co-solvents and surfactants are most often the active agents used in flushing solutions [65]. *In situ* flushing has been used to treat soils and aquifers contaminated with halogenated volatiles, nonhalogenated semivolatiles, and nonvolatile metals [66]. The technology can encounter various problems stemming from the flushing agent. Flushing solutions can adhere to the soil or the aquifer matrix, accelerate microbial growth, and cause dissolved constituent precipitation within the porous matrix and thereby reduce system permeability. Furthermore, difficulties can occur with separating co-solvents and surfactants from the elutriate. Left unaddressed, the above problems will manifest themselves in the form of greater demands for reactive agents and additional costs to treat and dispose waste fluids [66 and citations within]. However, such is not the case for flushing solutions comprised of HS: first because the cost of materials is low, and second because humics are biologically recalcitrant and not expected to support microbial growth. However, the use of humics does present its own challenges. For example, under certain conditions dissolved humics can adhere to soils and aquifer materials, and as a consequence, undermine efforts to flush by intercepting and immobilizing soluble contaminants. For a case study on the use of concentrated HS solutions for flushing technologies see review of Van Stempvoort et al. [67] and other related publications [68, 69].

Enhanced bioremediation is an active strategy whereby microbial processes are used to degrade or transform contaminants to less toxic or non-toxic forms. The technology specifically promotes microbial growth for the purpose of harnessing natural processes that effect direct and indirect contaminant degradation or transformation [70, 71]. Soils and aquifers contaminated with organic compounds such as petroleum hydrocarbons, volatile organic compounds, pesticides, wood preservatives, etc. have been treated successfully with bioremediation [72]. The technology has also been used to change the valence state of inorganics such as metal oxoforms for the purpose of inducing adsorption or uptake by microorganisms [73]. However, bioremediation can fail if the supply of nutrients, oxygen, or other electron acceptors is insufficient to support microbial growth. Furthermore, this type of remediation can be inhibited by high concentrations of contaminants or by the presence of other soluble constituents toxic to microorganisms. Other conditions such as pH or the presence of dissolved constituents more amenable to biodegradation can also affect the rate and efficiency of remediation. If used to enhance bioremediation, humics function as reactive agents that are not susceptible to degradation or expected to undergo co-metabolism with target contaminants. HS can serve as extracellular electron shuttles and accelerate microbial redox reactions [41, 42]. Humic-based products can in some cases ameliorate contaminant toxicity by transforming pollutants into less-toxic forms or by sequestering them in a separate phase and reducing bioavailability. With toxicants sequestered, microbial growth is stimulated, and from this the formation of bound residues may be intensified resulting in contaminants covalently bonded to humics [74-77]. Additional discussion on the detoxifying properties of humics is given later in this chapter. For a case study on intensified humification of TNT, see Thomas & Gerth [78].

Phytoremediation uses plants to intercept, accumulate, and/or degrade contaminants in soil and groundwater [79, 80]. The technology is applicable to a broad range of contaminants including numerous metals and radionuclides, various organic compounds (such as chlorinated solvents, petroleum hydrocarbons and their monoaromatic components benzene, toluene, ethylbenzene and xylene (BTEX), polychlorinated biphenyls (PCB), PAH, pesticides, explosives, nutrients, and surfactants). The technology requires plants which have specific characteristics including; tolerance to elevated contaminant concentrations; the tendency to produce significant root biomass; the capability of immobilizing contaminants through uptake, precipitation, or reduction; and the characteristic of retaining target contaminants within the roots such that special handling and disposal of shoots may be avoided [81, 82]. In this context, the application of humics-based products poses several advantages, because as indicated above, HS are known to reduce the toxicity of contaminants and increase a tolerance of the plants to chemical stress [83-85]. In addition, they stimulate the development of roots [86, 87] and as a result, bring about a desirable increase in root biomass. For more details on the direct effects of HS on plants, see reviews by Kaschl & Chen [88] and Kulikova et al. [89].

Clearly, a case can be made for the use of humics and humic-based products to enhance chemical and biological *in situ* remediation. However, in order to develop systematic humics-based applications, the properties of HS must be studied in the context of each remedial technology. In the following sections the fundamental interactions between humics, ecotoxicants and living organisms in the polluted environments are considered.

3.2. IMPACTS OF HS ON SPECIATION AND BIOAVAILABILITY OF ECOTOXICANTS

As indicated above, the broad-spectrum reactivity of HS exists because the humic structure contains numerous functional groups and hydrophobic moieties. This enables humics to bind with both metal ions and organic chemicals. The general binding of HS to ecotoxicants in homogeneous system can be described by the following formalized equation:

$$\mathrm{HS} + \mathrm{ET} \leftrightarrow \mathrm{HS{\cdot}ET} \tag{1}$$

The equilibrium constant *K* is commonly used to characterize this interaction:

$$K = \frac{[\mathrm{HS\text{-}ET}]}{[\mathrm{ET}]\times[\mathrm{HS}]} \tag{2}$$

where [HS], [ET], and [HS-ET] represent equilibrium concentrations of the reagents and the reaction product.

Due to the stochastic nature of humics, the stoichiometry of interaction (1) is unknown; hence, specific assumptions are introduced to facilitate the use of eq. (2). In the case of organic compounds, the most common approach is to treat humic substances as “dissolved sorbents” and the HS-ET interaction as a phase partitioning [90-92]. This approach assumes the equilibrium constant *K* equates to a partition coefficient characterizing a sorbate-sorbent interaction in a heterogeneous system. To account for the mass:volume ratio, the partition coefficient is normalized to the mass concentration of humics in solution, thus:

$$K_{OC} = \frac{1-\alpha}{\alpha} \times \frac{1}{C_{HS}} \tag{3}$$

where K_{OC} is a partition coefficient of ET normalized to mass concentration of soluble HS; α is the portion of the freely dissolved ET in the presence of HS, $\alpha = [\mathrm{ET}]/\mathrm{C}_{\mathrm{ET}}$; and C_{HS} is a mass concentration of HS expressed on an organic carbon basis (kg C/L).

From eq. (3), K_{OC} can be found by determining the fraction of the freely dissolved ET in the presence of HS. This can be done using common analytical techniques with or without preliminary separation of the freely dissolved and the HS-bound species of ET [93-97]. The reported K_{OC} values for polycyclic aromatic hydrocarbons (PAH) vary from 10^2 to 10^6 L/kg C [35, 36, 98-101]. The largest values of 10^5-10^6 L/kg C are observed for partitioning of PAH having four and more rings in their structure (pyrene, benz(a)pyrene, fluoranthene, and others) [101-103]. Much lower binding affinity is observed for small polar molecules like triazines, anilines, phenols, etc. [104-107]. In addition, a very strong dependence of binding affinity on the structural properties of humics is worth noting. When compared to aquatic humic or fulvic acids, considerably higher K_{OC} values are observed for humic acids derived from soil, peat and coal or for commercial humates enriched with aromatic moieties [100, 101, 108]. These findings are indicative of hydrophobic binding that is therefore a governing mechanism of interactions between humics and organic ET. The aromatics enriched humics from coal and mollisol are generally the most hydrophobic; consequently, they are more likely to bind organic contaminants than HS derived from other sources. This association between enhanced binding affinity and enriched levels of aromatic moieties within the humic structure was confirmed by the quantitative structure-activity relationship (QSAR) studies [101, 104, 109, 110].

The binding interactions with ET are of particular importance in remediation, as such interactions reduce concentrations of freely dissolved ET; and as a result leave the offending contaminant less available and perhaps less toxic to living organisms. This is shown in studies of the bioaccumulation [109, 111, 112] and the toxicokinetics [113-115] of hydrophobic organic contaminants in the presence of dissolved humics. For example, the bioconcentration factor (BCF) of PAH in the presence of HS is directly proportional to the fraction of freely dissolved PAH [109, 116]. Furthermore, the partition coefficients determined from bioaccumulation matches those measured by equilibrium dialysis. Similar results are also obtained in acute toxicity studies of three PAHs (pyrene, fluoranthene and anthracene) and a wide range of HS samples from water, soil, peat and coal [117]. Hence, the partition coefficients determined by analytical methods can be used as reliable predictors of the capacity of humics to bind and detoxify (or sequester) organic ET in aquatic environments. This is further confirmed by QSAR-studies revealing a direct correlation between soluble humics aromaticity and the ability to detoxify and/or sequester target hydrophobic contaminants e.g., PAH [109, 117]. In contrast, similar studies show the detoxification effects of HS are less consistent with polar organic compounds [118-120].

The discussed impact of binding interactions on bioavailability of organic ET is even more pronounced where metals are concerned. As in case of organic ET, metal toxicity is related to the free aqua metal ion concentration rather than the total metal concentration. The binding of heavy metals to HS causes a change in metal speciation followed by a change in toxicity and bioaccumulation [121]. This is confirmed by a number of publications (see review [122] and the related recent publications [85, 123-128]).

Figure 5 conveys the concept that HS can bind with ET and as a result, reduce the bioavailability and the toxicity of ET. As indicated in Fig. 5, contaminant detoxification is most relevant in biologically based technologies such as phytoremediation and with *in situ* bioremediation. Depending on the contaminant, elevated concentrations can be toxic to microorganisms and plants and as such undermine remediation efforts unless local concentrations are reduced. The function of humics in this case is simply to reduce concentrations of freely dissolved ET while bioremediation or phytoremediation resumes. Thus, in the case of hydrophobic organic ET, hydrophobic humics from coal are likely to be more effective than HS extracted from other sources. This is an important practical outcome of the above noted QSAR studies. In case of metals, the structure-activity relationship is much more complex, and desired results may depend on the availability of "designer" humics or humics customized to enhance metal complexing properties; the concept of designer humics is discussed further in the final section of this chapter.

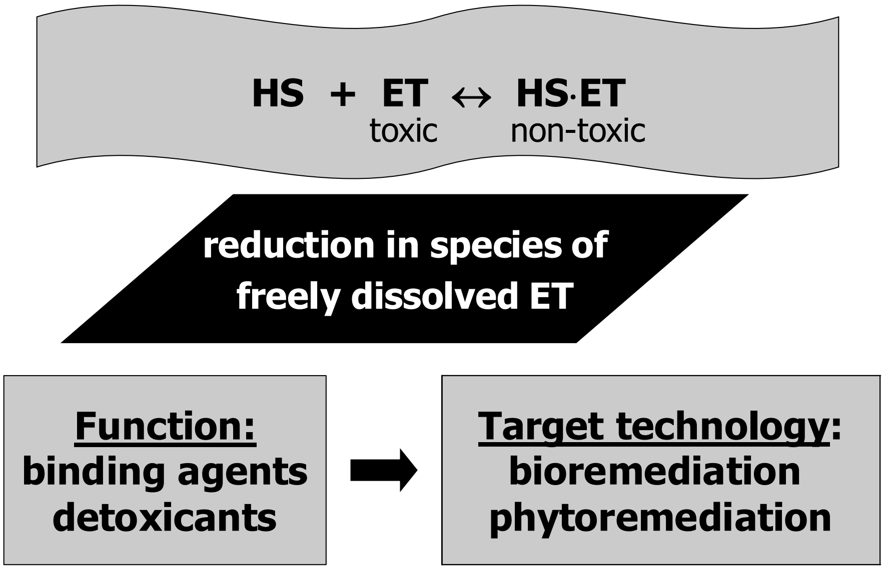


Figure 5. HS can bind ecotoxicant (ET) and as a result reduce the bioavailability and the toxicity of ET. This concept has immediate relevance in biologically based remediation technologies such as *in situ* bioremediation and phytoremediation.

An extremely important issue constraining the application of humic substances in remediation is the unknown stability and longevity of humic complexes and/or adducts with ecotoxicants under environmental conditions. Clearly, quantitative studies on the dissociation kinetics of HS complexes with organic chemicals and heavy metals are particularly important for the proper evaluation of humics as reactive materials for PRB and other technologies. Similarly, the same is true of detoxification, long-term experiments are needed to evaluate humics as detoxicants. All investigations regardless of focus (i.e., humic/ET complexation, partitioning, or detoxification), need to be conducted under a range of environmental conditions and using a broad variety of humic samples.

3.3. IMPACTS OF HS ON THE INTERFACIAL INTERACTIONS OF ECOTOXICANTS WITH MINERALS

The previously discussed binding of ET to HS in an aqueous system is often treated as a homogeneous reaction between a dissolved contaminant and a dissolved humic macromolecule. In the soil and the subsurface, however, the leading interaction is simply the heterogeneous sorptive partitioning of ET between the water and the solid phase. The water phase contains soluble inorganic ions and dissolved humic components, whereas the solid phase is represented by minerals and organo-mineral complexes. The latter are formed due to the sorption of humics onto mineral surfaces; the resultant humic coating functions as a natural sorbent with regard to contaminants. Hence, when fixed on mineral surfaces, HS can retard migration of trace metals and organic contaminants; but when dissolved in water, humics can facilitate the transport of the contaminants in the subsurface. Both processes are intensively discussed in the literature. For example, immobilization on organo-mineral particles has gathered considerable attention among researchers investigating the migration of hydrophobic organic contaminants (HOC) in soils and sediments [129], while facilitated transport with organo-mineral colloids has been the focus of the studies concerned with the subsurface migration of heavy metals and radionuclides [130, 131].

The immobilization of HOC by humic coatings recently captured the interest of scientists and engineers when it was shown the binding affinities for HOC were several orders of magnitude higher for humics immobilized on sediments compared to those dissolved in water [98, 132-134], and that the sorption capacity for HOC was proportional to the soil/sediment organic carbon mass fraction [135]. Typically, linear, equilibrium partitioning models are used to quantifying organic ET sorption [135]. These models employ a distribution coefficient K_D to describe the partitioning between aqueous–phase and solid-phase concentrations of ET at equilibrium. As in the homogeneous system, the coefficient K_D is normalized to the soil/sediment organic carbon mass fraction, (f_{OC}), to yield a relatively constant partition coefficients $K_{OC} = K_D / f_{OC}$ for a given ET. Furthermore, as previously indicated, the sorption or partitioning is assumed to be linear, instantaneous, and reversible, that is not subject to competition among different HOC solutes. However, reported findings on HOC sorption by organo-mineral complexes reveal: sorption is indeed characterized by substantial non-linearity and hysteresis [136-140]; and that the sorption affinity of bound humics is more complicated than the simple structure-property relationships revealed by QSARs for homogeneous systems [141-143].

To explain the non-ideal sorption phenomena and the complicated character of the QSPRs, a dual reactive domain model was developed and introduced almost simultaneously by Weber and his group [144] and Xing and Pignatello [145]. The formulation of this model assumed the organic (humic) material bound to mineral surfaces was comprised of two principal organic domains: one a highly amorphous domain (rubbery domain) and the other a relatively condensed domain (glassy domain). Sorption of HOC coupled to the amorphous domain was linear, fast and completely reversible; thus, it could be described using the linear equilibrium partitioning model. However, in the glassy domain, sorption was slow, non-linear and hysteretic [146].

Numerous studies have since validated the two-domain model. However, disparate opinions exist in the literature as to the structural moieties (aliphatics or aromatics) responsible for the hysteretic sorption observed in the glassy domain. A number of recent publications [147-150, and citations in them] claim previous studies have overestimated the importance of the aromatic moieties present in humics and most specifically with regards to explaining the sorption of hydrophobic ET. Simultaneously, these contemporary investigators have espoused the concept, that humics possess an aliphatic component which plays a major role in controlling the sorption of organic contaminants. This assessment is predicated on results of comparative sorption studies involving non-polar probes (phenanthrene, pyrene, etc.) and an array of sorptive matrices including polymers enriched with aliphatics (as opposed to aromatic structures) and different humic fractions. The aliphatics thesis has acquired additional support from solid-state CPMAS ^{13}C NMR data gathered on the presence of poly(methylene)-rich aliphatic domains in the different humic fractions and from correlations developed between aliphatic moieties and non-polar organic sorption [151, 152]. However, it must be noted that in these studies substantial differences exist between the organic matter found in humic coatings and the solid humics used in the NMR studies. The former represents heterogeneous surface complexes of minerals and organic macromolecules, whereas the latter represents a condensed polymeric phase. Hence, the discussed poly(methylene) aliphatic domains were detected in the condensed polymeric phase of humics, and as such caution should be exercised with regards to extrapolating their existence in humic coatings on mineral surfaces. Of course, insight into this question could be provided by conducting analogous NMR studies of model humic-clay complexes obtained using well-characterized humics.

From the above considerations, the following strategies can be formulated for the use of HS in remediation. Humics enriched with glassy rigid domains in their structure (supposedly, rich in aliphatics) are preferential in applications as reactive materials in sorptive barriers designed to intercept and retain non-polar organic ET. The corresponding concept is shown in Fig. 6. These humics have the highest sorption affinity for organic ET and provide the slowest desorption kinetics, or the highest retardation of the organic ET. In terms of the "designer" humics, the best candidates for a use as HOC-sorbents would be cross-linked humics rich in rigid or glassy domains. A potentially cost effective method of creating humic-based sorptive PRBs is to construct them without excavation, in other words, use an *in situ* process of attaching humics to the aquifer matrix. G. Balcke et al. [64] are among the first to investigate an *in situ* approach of coating mineral surfaces with injected humics. QSAR studies provide considerable insight into the adsorption mechanism responsible for the adherence of humics onto mineral surfaces [153]. These studies show the highest affinity for mineral surfaces is seen for humics enriched with aromatics, and that sorption reversibility is inversely proportional to the molecular weight of the humics used. Hence, aromatic-rich humics of high molecular weight are likely to be the best candidates for producing reactive coatings on mineral surfaces.

Illustrated in Fig. 7 is yet another process whereby an ecotoxicant bound to organic-mineral complex is mobilized due to the formation of adducts with dissolved humics. The surfactant properties of HS can be used to develop humic-based flushing agents suitable for the *in situ* remediation of soils and aquifers contaminated with hydrophobic organic chemicals. Aromatics-rich dissolved humics could be the best candidates for this purpose. The reduction of ET concentrations in groundwater will cause a shift in the partition equilibrium towards groundwater and ultimately result in total contaminant removal. Van Stempvoort et al. [67] present a case study on organic ET mobilization using concentrated Aldrich humic acid solutions.

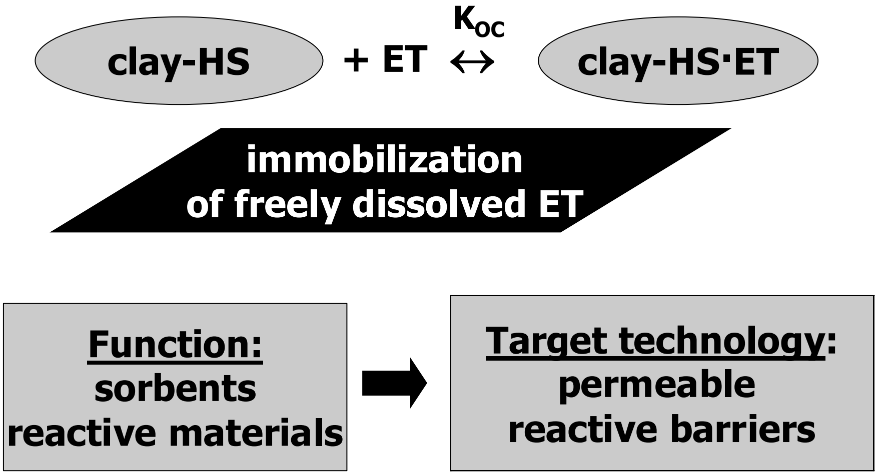


Figure 6. Sorption of ET by the stationary HS-mineral complexes effectively retards the transport of ET in groundwater. This concept can be extended to the design of PRBs where HS-mineral complexes comprise the surfaces of reactive materials and function to intercept and retain mobile ET from groundwater.

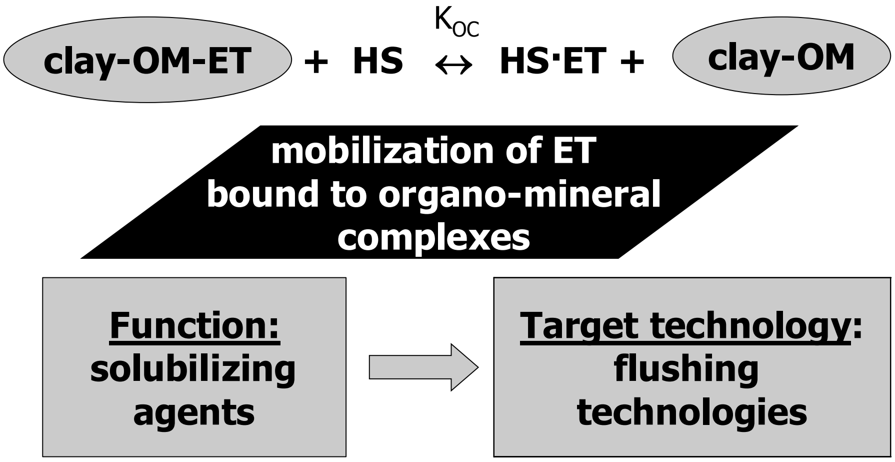


Figure 7. Dissolved humics form HS-ET complexes and enhance ecotoxicant desorption from the mineral surfaces. Under this scenario, HS solutions may be used as flushing agents to facilitate transport of contaminants through an aquifer.

Another application of the surface active properties of concentrated solutions of HS is to facilitate the flow of water through hydrophobic zones of contaminated aquifer. For example, in areas contaminated with residual immiscible hydrocarbons and dense non-aqueous phase liquids the aquifer matrix is sufficiently hydrophobic that the flow of water through the medium is inhibited. Humics introduced into the mobile water phase will reduce the interfacial tension at the surface of porous matrix and permit the aqueous phase to penetrate the hydrophobic medium. With nutrients and electron donors/acceptors supplied, local indigenous microorganisms are stimulated to degrade contaminants. Thus, it is not always necessary to mobilize the contaminant; rather, in this case the main goal is to facilitate the penetration of groundwater, laden with nutrients and electron donors/acceptors, into the contaminated hydrophobic soil matrix.

3.4. IMPACTS OF HS ON THE METABOLISM OF ECOTOXICANTS DUE TO ABIOTIC AND BIOTIC REDOX MEDIATION

Many ecotoxicants such as petroleum hydrocarbons, their monoaromatic components (e.g., BTEX), hydrazines and amines are highly reduced. Hence, oxidation is the primary path of degradation. On the other hand, many contaminants are highly oxidized such as chlorinated hydrocarbons, nitroaromatics, and anions of transition metals, and for these pollutants reduction is the feasible pathway of terminal transformation. Reported values of formal electrode potentials for HS vary from +0.15 to +0.79 [28, 154-158]. From this range and the reversibility of redox transformations, it may be surmised that the redox properties of humics are attributable to the quinonoid moieties present in the aromatic core [159]. Moreover, direct electrochemical evidence exists on the quinonoid nature of the redox-active units [160]. Natural organic matter (NOM) (particularly, the polyphenol fraction) gives an electrode response similar to that of model quinones such as juglone, lowsone, anthraquinone disulphonate (AQDS). Hence, similar to quinones, humics can participate both in abiotic and biotic redox transformations of ET in contaminated environments.

Several studies can be cited where HS were shown to participate in abiotic redox transformations. For example, direct abiotic reduction of Cr(VI) by HS was reported [161-164]. In addition, reduction of highly oxidized actinides such as Pu(VI,V) and Np(VI) also has been demonstrated [165, 166]. However, U(VI) and Np(V) reduction was not observed with HS of natural origin [167]. The customized humic materials of the enhanced reducing capacity can be of particular value to serve that purpose. Their synthesis is discussed further in this chapter. Another example of an abiotic redox reaction where transition metal complexes of HS catalyse the abiotic reduction of a priority pollutant is reported by O'Loughlin et al. [168]. They showed that Ni-HS complexes effectively enhanced the reduction of different chlorinated hydrocarbons in the presence of Ti(III) citrate as the bulk reductant. Similar catalytic effects were caused by Cu-HS complexes. Hence, humics represent potential reactive materials for the immobilization of highly oxidized species of radionuclides and heavy metals, and for the reduction of highly oxidized organics (Fig. 8).

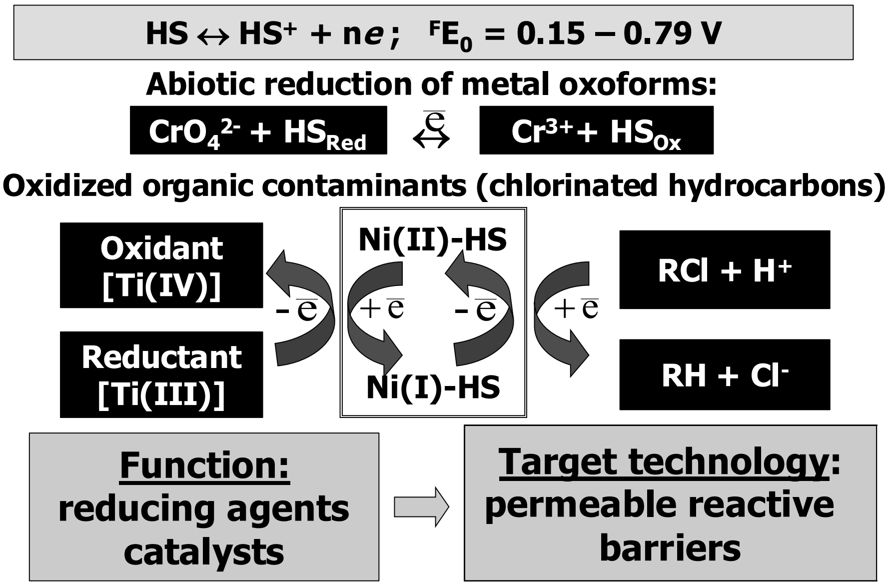


Figure 8. Reported values of formal electrode potential for HS vary from +0.15 to +0.79 [157, 158]. Over this range HS can facilitate both direct and indirect abiotic reduction of highly oxidized contaminants. The given reactions demonstrate direct abiotic reduction of oxoforms of high valence metals on the example of Cr(VI) [161-164] and show mechanism of catalytic impact of transition metal complexes of HS on kinetically slow abiotic reduction of organic contaminants by the bulk reductant as reported by O'Loughlin et al. [168].

Recently, considerable attention has been focused on the ability of humics to mediate the microbial degradation of various contaminants (see review [169]). Humics possess the unique capability of functioning as both an electron acceptor and a donor depending on environmental conditions [41, 42]. This ability permits humics to facilitate both oxidative and reductive biodegradation as is shown in Figs. 9 and 10. Under anoxic conditions, humics operate as terminal electron acceptors supporting the mineralization of various organic pollutants to CO_2 by anaerobic microbial communities [44, 170, 171]. Fig. 9 illustrates the feasibility of HS functioning as redox mediators within a technology designed to bring about *in situ* oxidative bioremediation [56].

It has also been widely reported that HS facilitate reductive biodegradation by shuttling electrons from microorganisms to various highly oxidized organic contaminants (e.g., chlorinated hydrocarbons and azo dyes [172-174] as well as to high valence metals (e.g., Cr(VI), U(VI), and Tc(VII)) [43, 175, 176]. Fig. 10 reveals that HS can mediate reductive biodegradation both directly via shuttling electrons from microorganisms to high valence metals or oxidized organics, and indirectly via interactions with different Fe(III) oxide minerals [177]. Hence, it is plausible for humics to function as redox mediators within technologies designed to bring about *in situ* reductive bioremediation [177].

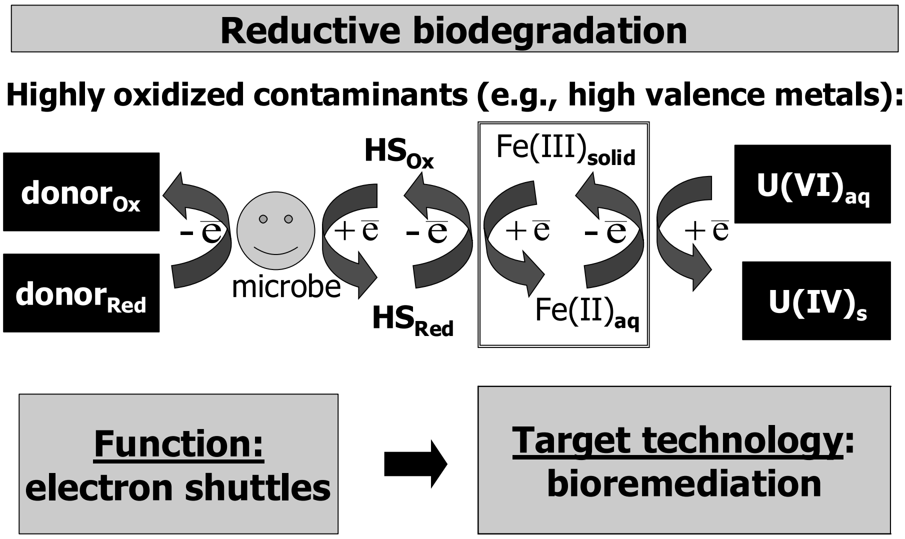


Figure 9. Under anoxic conditions, humics function as terminal electron acceptors or redox mediators supporting the oxidative biodegradation of the reduced organic pollutants to CO_2 by anaerobic microbial communities. An example of the corresponding technological development is published on the site of the USGS [56].

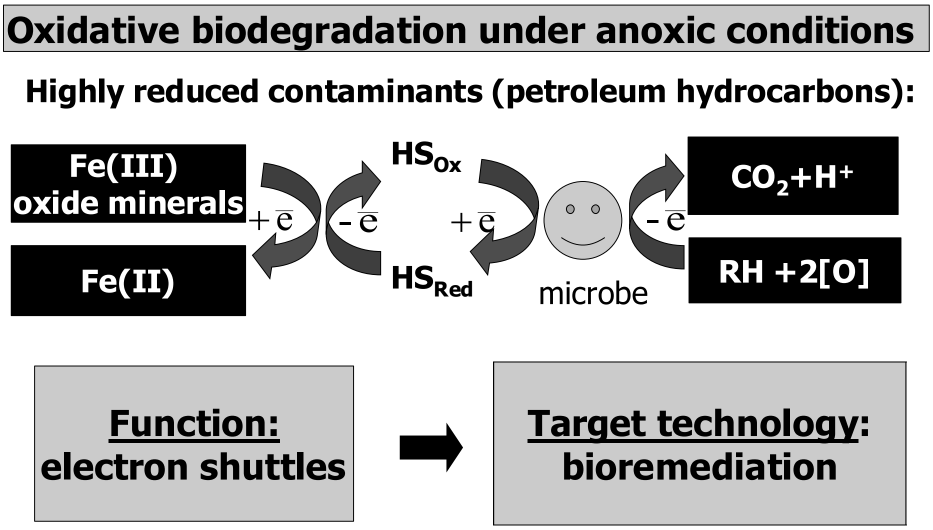


Figure 10. HS can participate in the reductive biodegradation of various highly oxidized contaminants (i.e., metal oxoforms or chlorinated hydrocarbons) either by direct shuttling electrons from microorganisms or via interaction with Fe-oxides. Enhanced bioreduction of U(VI) was described in [43]. Operating as redox mediators, it is plausible for HS to function in technologies designed to bring about *in situ* reductive bioremediation [177].

Given the above described electron shuttling properties, it is conceivable humics can be used to facilitate or stimulate a suite of microbially mediated redox reactions pertinent to *in situ* bioremediation. However, the primary factor limiting their effectiveness is the structural polydispersity and heterogeneity which translates into reactive properties that are highly variable between natural sources and amongst different humic fractions. Gu and Chen [43] revealed a wide disparity in redox mediating properties among humic samples of different origin and fractional composition. For example, the best performance in the abiotic reduction of Cr(VI) and Fe(III) are observed with a polyphenols-rich fraction of natural organic matter (NOM). However, soil humics enriched with polycondensed aromatic moieties are more effective in mediating the microbial reduction of Cr(VI) and U(VI). To overcome the problem of structural heterogeneity and polydispersity, directed modification of HS may be advantageous, e.g. the incorporation of additional quinonoid moieties to bring about a desired enhancement in redox-capacity [178].

Finally, it is necessary to point out that HS exhibit yet another kind of mediating effect on the biotic transformation of organic ET; this is the covalent bonding to humics also known as oxidative coupling [179]. This process includes the oxidation, generation, and rearrangement of free radicals, and the incorporation of the ecotoxicant into the HS structure. To introduce oxidative coupling into the practice of remediation, biostimulators of this process must be designed. Early efforts to initiate oxidative coupling with low molecular weight initiators (substrates of oxidoreductive enzymes) proved inefficient [180]. An alternative approach, using high-molecular weight humics-based promoters deserves further consideration. These promoters can be prepared by directed modification of humic materials, given the properties of such materials are clearly defined by environmental microbiologists.

3.5. IMPACTS OF HUMICS-ECOTOXICANTS INTERACTIONS ON LIVING ORGANISMS

The pool of data describing the direct biological effects of HS is vast, miscellaneous, and controversial. The array of biotargets studied includes pure cell cultures, bacteria, algae, fungi, higher plants, animals, and humans. Some authors show humics stimulating the growth of higher plants [51, 181-183] and microbial communities [184-186]. Other investigators find HS strengthen the resistance of higher plants under stress [50, 52, 87] and as a consequence, define humics as natural adaptogens. Mazhul et al. [187] and Vigneault et al. [188] believe humics have a direct impact on cells by changing the permeability of the cell membrane; whereas others claim HS increase the bioavailability of nutrients via the formation of metal-HS complexes [181, 189].

Given the growth stimulating and adaptogenic (anti-stressor) activity of humics, these substances may be useful as agents to enhance bioremediation and, in particular, phytoremediation. Fig. 11 conveys a concept of humics use in phytoremediation technologies. The beneficial effects of HS on plant growth in the polluted environment can be related to an increase in nutrient supply, to an improvement of the overall plant development, and to an increased resistance to chemical stress [190, 191]. In the context of phytoremediation, it is well documented that humics cause an increase in root growth superior over that of the shoot [192]. Taken together, the combined effects humics suggest they are engaged facilitating nutrient transport, promoting plant growth, and serving as bioadaptogens.

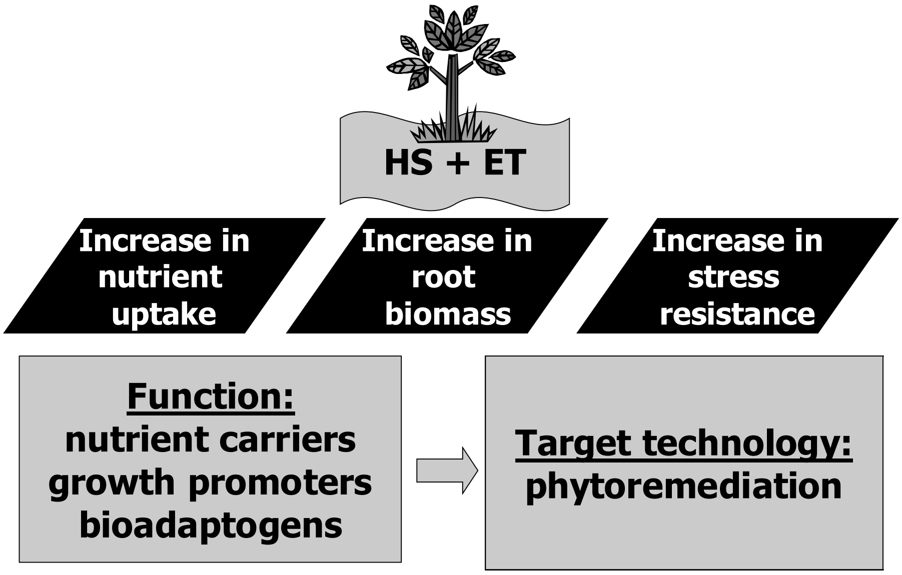


Figure 11. Potential functions performed by HS for higher plants in the polluted environments and their associated biological responses.

Existing studies on quantitative-structure activity relationships for beneficial effects of humics on higher plants deliver contradicting results [51]. The most consistent are the studies on the effects exerted by high and low molecular weight humic fractions. It is shown that high molecular weight fractions promoted the plant growth, but decrease enzyme activity [86, 193]; accelerate root differentiation [194]; and readily adsorb onto the cell wall, but do not enter the cell [195]. At the same time, low molecular weight fractions were shown to reach the plasmalemma of root cells and, in part, were translocated into the shoots [196]. Such observations brought Nardi with co-workers to review the physiological functions of HS on higher plants [51] and to conclude that it was the lower molecular weight humic fractions that acted at the symplast and directly influenced plant metabolism, whereas the higher molecular weight fractions operated mainly on the cell surface where they influenced differentiation and growth at the apoplast. Hence, the reported positive effects on plant growth were induced by the lighter fractions of humic matter.

The conditions of polluted environment require meticulous consideration, when rationalizing the use of humics to enhance phytoremediation. Another recent review on molecular size dependent impacts of humics-ecotoxicants interactions on biota by Perminova et al. [122] should be particularly mentioned here. This review demonstrates that the adverse effects of the ecotoxicants are always lower if those contaminants possessed a strong affinity for humics representing the higher molecular weight fraction. Whereas for ecotoxicants having a greater affinity for lower molecular weight fraction, toxicity can decrease, remain the same or increase. It is hypothesized that for humics of high molecular weight, the complex with the ET forms that cannot penetrate the cell membrane and, hence, reduces bioavailability of the ecotoxicant. In case of humics of low molecular weight, the corresponding complex (or associate) is translocated to the interior of the cell where transformations of the humic-ET complex occur under the prevailing conditions inside the living cell interior. If the complex (associate) does not dissociate under cellular conditions, no additional toxicity occurs. However, if the complex (associate) breaks down, the toxic effects of the ET will be enhanced. Typical contaminants likely to share a high affinity for the heavier humics are: HOC like polycyclic aromatic and polychlorinated hydrocarbons, and metals like Cu and Pb. ET among the group most likely to bind with lighter humics are: trace metals and organic chemicals which bind to humic molecules via ion exchange or hydrogen binding, or the other mechanisms involving not the core but functional moieties of the humic compound. Examples of this group of ET include cadmium, Cr(VI), substituted phenols/anilines, and others.

Hence, to ensure positive biological effects of HS on higher plants in the polluted environments the preference should be given to a use of humics of higher molecular weight. More research on the biological effects of well characterized HS is needed to develop sound scientific base for humics applications in phytoremediation.

4. Design of humic materials of the desired properties, or how to make humics work for remediation technologies

Despite the diverse protective functions transferable to humics in a polluted environment, application of humics-based products for remediation remains limited. Two fundamental reasons can be formulated as to why HS are not widely used. First, few natural HS possess the specific reactive properties required to treat selected contaminants. Second, humics by definition are polydisperse and heterogeneous, which translates into properties that vary between natural sources and between industrial suppliers. Hence, the structural heterogeneity needs to be reduced or controlled to the extent that reactive properties become predictable; this will facilitate the use of humic materials in remediation. From this perspective, Perminova and co-workers are developing “designer humics” in other words, they are chemically modifying humic materials to acquire desired reactive properties [178, 197].

Fig. 12 conveys a conceptual model for designing reactive humic materials based on idea of reducing structural heterogeneity and polydispersity. This model introduces the concept of incorporating specific reactive moieties into the humic backbone for purposes of acquiring desired reactive properties, and of cross-linking humic materials for producing a desired reactive form (soluble, colloidal, solid).

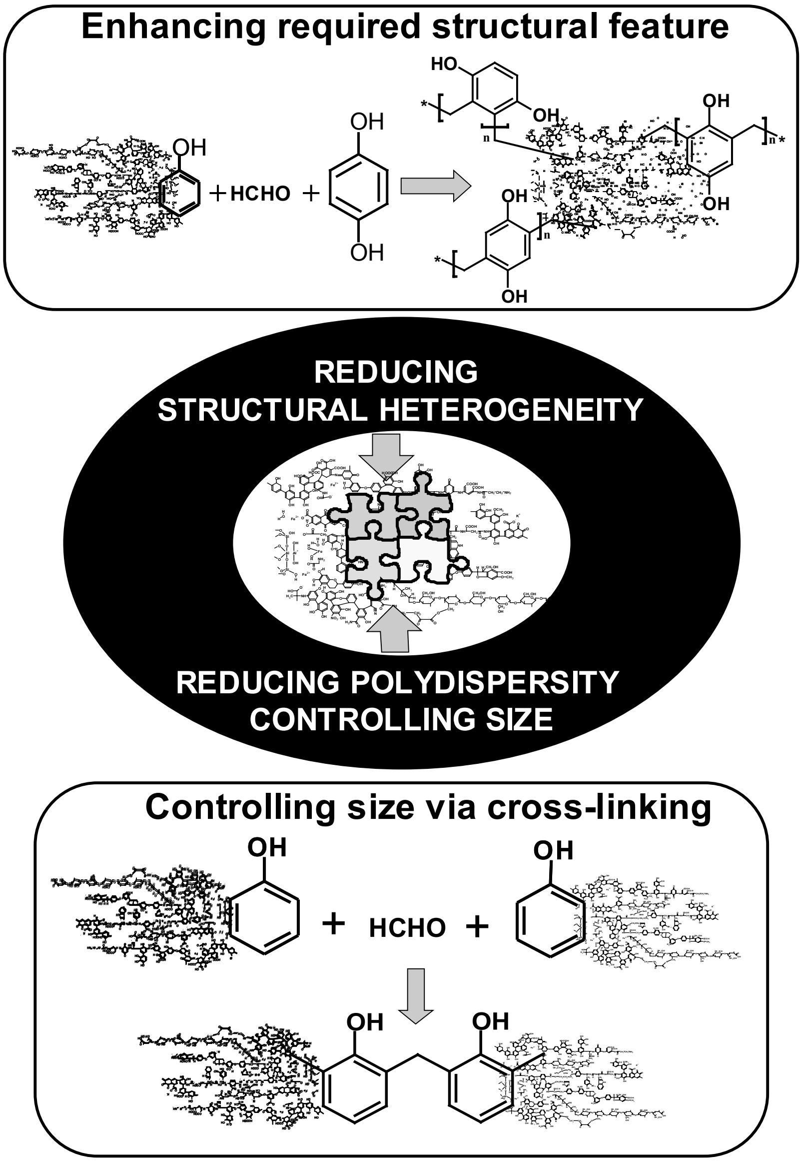


Figure 12. Conceptual model of the design of humic reactive materials. The examples are shown for tailoring redox-active and cross-linked structures.

using phenolformaldehyde-like condensation reactions with coal-based HS, and hydroquinone and catechol monomers (see Fig. 12). The modified humics possess reducing capacities with respect to Fe(III) that are greater than parent humic materials [178]. Cathecholic derivatives are more effective at detoxifying Cu(II) [198] than humics modified with hydroquinone monomers; and this confirms the formation of non-bioavailable Cu(II) chelates as the main mechanism of detoxification. The demonstrated consistency of the changes in structure and properties of the humic derivatives lay bare the feasibility of employing chemical modification for preparing humic materials of the desired properties. For example, an available supply of humics possessing a variety of quinonoid reactive centres and covering a wide range of electrode potentials presents a unique opportunity to probe a selection of electron shuttling interactions mediated by humics between ecotoxicants and diverse microorganisms. As a result, a strategy can be formulated for a use of humics as biostimulators to accelerate the microbial redox transformation of the contaminants.

The different physical forms of the designed humics - soluble, colloidal, and solid, - can ensure control over interfacial interactions of HS with contaminants. The soluble forms can be used either for retarding heavy metals and radionuclides migration in soil or as biostimulators of metal reducing microbial communities. The colloidal and solid forms may be embedded into permeable polymer matrixes (e.g., polyurethane foams) for purposes of producing easily deployable sorbents suitable for vehicle and building decontamination. Solid forms can also be used as sorbents for PRBs or they can be applied to other forms of engineered flow-through systems. It is feasible that humic reactive materials be developed to suit dual needs of both site remediation and remediation monitoring. In particular, humics can be used as sorbents and reactive materials in novel strategies to quantify remediation performance and to better characterize remediation potential as described below.

Hatfield with co-workers [199, 200] present a passive flux meter (PFM) method for measuring *in situ* groundwater flux and contaminant mass flux. The PFM is a self-contained permeable unit that is inserted into a well. The interior composition of the flux meter is a matrix of hydrophobic and hydrophilic permeable sorbents. As ground water flows passively through the device, the internal sorptive matrix retains dissolved contaminants present in the volume of water intercepted. The total mass of contaminant intercepted and retained on the sorbent permits direct determination of the local *in situ* contaminant mass flux. The latter yields a quantitative assessment of the source loading or strength, – a change of which is the primary indicator of the effectiveness of remediation. Fig. 13 illustrates the PFM as simply a permeable cartridge that is inserted into a well located down gradient from a contaminant source. The sorptive matrix is also impregnated with known amounts of one or more water-soluble resident tracers. These tracers are displaced from the sorbent at rates proportional to the groundwater flux; hence, the resident tracers are used to quantify groundwater flux.

To enhance characterization of biological subsurface remediation, humics can be used to create new sorbents for a new class of PFM’s designed to measure microbial mass fluxes. Novel humic-based sorbents serve to intercept and retain microbial biomass. Used in conjunction with the traditional PFM, the passive biomass flux meter

(PBFM) offers an opportunity to better characterize spatial variations in microbial biomass and ecology - indicators of bioremediation.

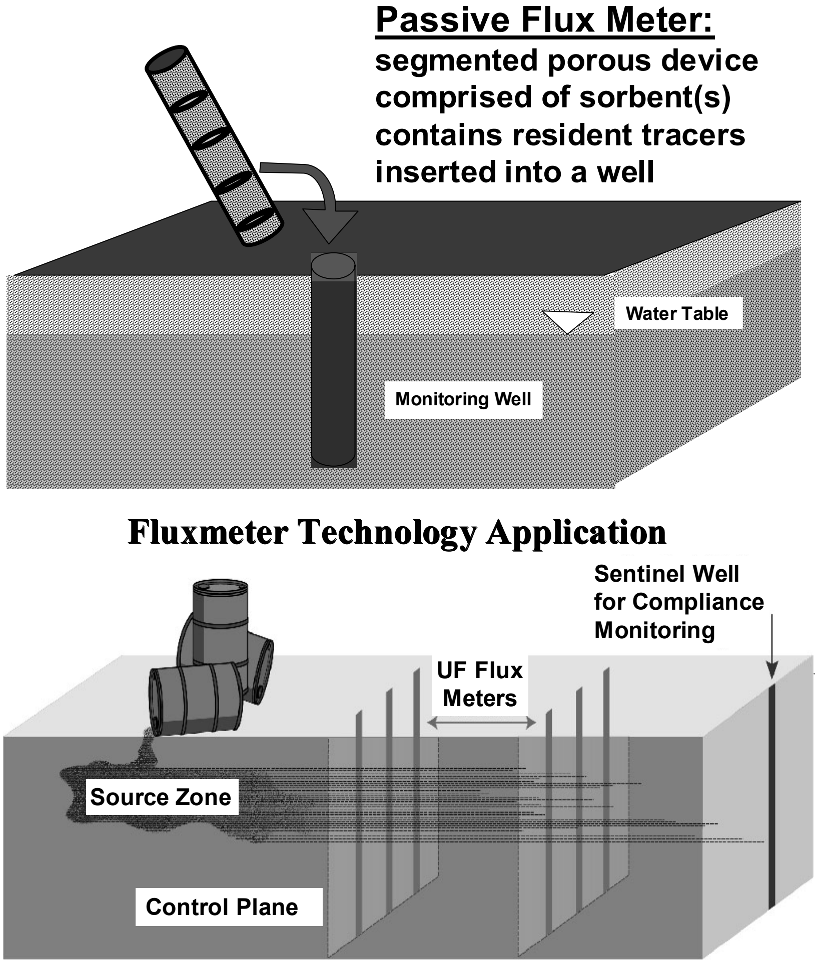


Figure 13. Conceptual model and application of flux meter technology.

Given the biocompatibility of humics together with their resistance to biodegradation, solidified humic matrixes represent ideal sorbents for bacteria and can be inserted into a monitoring well with little concern for possible negative repercussions. Hence, modified humics combined with passive monitoring methods presents new opportunities for the combined chemical and biological characterization of subsurface remediation.

5. Conclusions

An overview of the interactions of HS with ecotoxicants and living organisms in the polluted environments in the context of remediation chemistry allows us to conclude that HS can perform perform multiple functions within a remedial strategy. For example, it is shown that HS can function as: binding agents and detoxicants; sorbents and flushing agents; redox mediators of abiotic and biotic degradation; and nutrient carriers, bioadaptogens and growth-stimulators. With the above functions characterized, it is then proposed that humic-based products hold great promise as reactive agents for in situ remediation. The most promising technologies include: enhanced bioremediation; permeable reactive barriers; *in situ* flushing; and phytoremediation. However, to develop successful humic-based applications, it is stressed that the properties of HS must be studied in the context of each remedial technology. For example, research is needed to define the kinetics, stability, and longevity of humic complexes and/or adducts with organic chemicals and heavy metals. Results from such research are salient for the proper evaluation of humics as reactive matrices for PRB's and other technologies.

In the final section of this chapter, a novel concept is introduced for the development and application of designer humics. For the most part, natural humics possess an elemental composition that is non-stoichiometric and a structure that is irregular and heterogeneous; consequently, reactive properties vary widely between humic samples from various sources. Using example methods of chemical modification described herein, it is proposed that the properties of humics be tailored to satisfy the needs of a given remediation technology. Pursuing this approach, it is expected that the utility and the value of humics will expand.

6. Acknowledgements

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