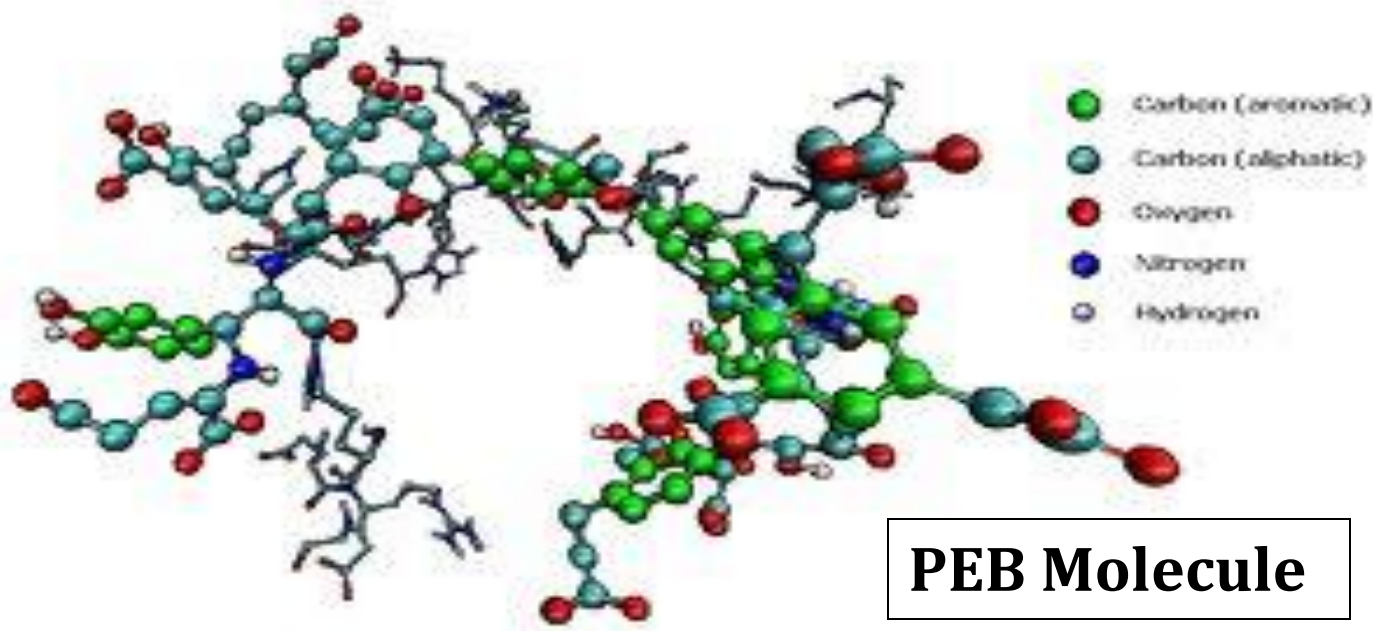


Scientific Base of the Technologies for Heavy Metals and Petrochemical Hydrocarbons Detoxification by Polyelectrolyte Enhanced Biopolymer

Polyelectrolyte Enhanced Biopolymer (PEB) is an organic substance in terms of its natural origin, and an organic-mineral product in terms of its chemical composition. It is derived from weathered organic matter. PEB is a 3-dimensional molecule and their associates which can be described in terms of biopolymers and polyelectrolytes.



PEB's polyelectrolyte part is a polymer with primarily aliphatic chains which include such oxygen bearing functional groups as carboxylic, carbonic, phenolic and alcohol hydroxyl groups. Protons (positively charged atoms of hydrogen) in these functional groups are available for ion exchange. Most of PEB's carboxylic groups and phenol/alcohol hydroxides are ionized resulting in a significant negative charge of the polymer and in its electrolyte properties. Polyelectrolytes (polymer-electrolyte) being negatively charged are extremely active in interaction with positively charged ions – cations through ion exchange mechanisms. Carboxylic groups (COOH) and phenolic groups (-OH) are the dominating functional groups in the ion exchange interaction with metals cations.

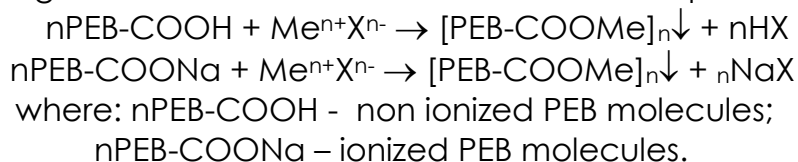
All of these actions cause PEB's high biological, geological and chemical activity and wide scale application in agriculture, industry, remediation, environment protection/restoration, etc. In these terms, PEB is a non-specific product due to its ability to positively affect many processes, substances, conditions, etc. PEB's most common application is for immobilization/detoxification of heavy metals in soil, waste water, industrial and municipal waste.



Note 1: Heavy metals are toxic in mobile forms as cations and exist primarily in aqueous media. Once immobilized/bound into insoluble compounds, heavy metals lose their toxicity. PEB is effective in decomposing different petrochemical hydrocarbons, including chlorinated hydrocarbons.

PEB is a highly soluble substance which can exist in liquid and solid form. PEB in liquid form is more active than in dry form because its volumetric molecules are already hydrated and hydrolyzed and all functional groups opened up for interaction in full strength/full ion exchanging capacity.

PEB ion exchange interaction with metals cations can be presented by the following:



$\text{Me}^{n+}\text{X}^{n-}$ - Me^{n+} - metal cation (for example, Ni, Zn, Cu, Pb, Hg, etc.) and X^{n-} - anion (for example, Cl, SO₄, NO₃, HCO₃, etc.).

This reaction results in soluble PEB molecules: nPEB-COOH and nPEB-COONa conversion into insoluble PEB-metals compounds $[\text{PEB-COOMe}]_n\downarrow$ and their precipitation. These precipitants contain immobilized and nontoxic metals.

Note 2: This is the same ion exchange as it is in the case of ion exchange resins. An ion exchange resin is a polymer with ion active functional groups. In most cases this is a solid insoluble granule or matrix with high porosity. For example, cation active resins release protons and adsorb metal cations. Once all functional groups are occupied with metals, the resin stops working and regeneration is required, i.e. metals replacement by protons for next resin use. Usually strong acid is applied for resin regeneration.

In summary, ion exchange in PEB binds, immobilize/stabilize and detoxifies heavy metals. PEB - metals are extremely stable. No microbial decomposition is possible. No metals leaching is possible at normal environmental conditions: soil pH, temperature, sunshine (ultraviolet) irradiation, moisture, bacteria, etc.

Only specific treatments can affect PEB-metals stability. For example, PEB-metals can be thermally decomposed at temperature around 600 C. Because ions exchange mechanisms are reversible, the heavy metals which were bound by carboxyl and phenolic groups can be extracted from PEB-metals by long time mixing (for several hours) with strong mineral acid such as hydrochloric acid, sulfuric acid, nitric acid or their mix at pH that is equal or below 1.5. In this case mineral acids protons replace heavy metals and turn them into soluble/mobile and respectively toxic forms – metals chlorides, sulfates, nitrates. But once again, such environmental conditions do not exist.

PEB's biopolymer portion is presented by long chain volumetric molecules with irregular structural units primarily of an aromatic nature which include such nitrogen bearing compounds as: Amino Acids; Amino-Sugars, Ammonium and Nitrogen- Hetero cycles; sulfur (sulfates, sulfonates, etc.) and oxygen (ester, ether, etc.) bearing groups with numerous electrically charged sites located inside volumetric molecules. There are also many hydrophobic and hydrophilic groups. These result in high absorptive capacity to both positively (metals cations) and negatively charged ions (anions) and lower molecular mass

compounds with electrically charged edges. Negatively charged groups of atoms dominate in PEB molecules and can actively interact with positively charged ions resulting in coordination complexes formation.

Note 3: *Coordination complex or metal complex is a type of bond between ions of metals and surrounding array of molecules or ions – ligands. PEB's molecules are the potent ligands. These result in a high complex forming ability for metals ions and specific groups of atoms which can pass and travel into 3-dimensional PEB molecular structures where they are bound/immobilized/stabilized.*

Note 4: *There is a “zero” balance between total positive charge of all metals cations and total negative charge of all anions (such as Cl, SO₄, NO₃, etc.) which can pass and travel into PEB. Because PEB's anionic groups dominate over cationic groups, metals cations binding into complexes results in total positive charge reduction and total increase in negative charge. In this case anions surround PEB-metals complexes and actively interact with PEB's positively charged sites. The more metals cations bound into complexes, the more anions bound into metals complexes as well and coordinated by PEB's positively charged sites. If metals cations do not form complexes, anions will not form complexes as well – however, a small amount of anions absorption by PEB's positively charged sites will occur.*

Multi valent metal cations interact with PEB molecules. This resulting “chelate” bonds forming insoluble PEB-metals chelates and precipitation. Insoluble precipitants are nontoxic. PEB-metals chelates is one form of metals complexes.

The heavy metal cations which entered into volumetric PEB molecules and are bound through complex formation with negatively charged nitrogen, oxygen, sulfur bearing groups, are not available for leaching by acidic treatment. Chelates also are not available for metals leaching by strong acids.

Note 5: *PEB molecule sizes and mass are pH dependent: the higher the PH the smaller the molecules and the lower the pH, the greater the molecules.*

Acidic media increases PEB molecular mass and size, forms PEB large molecular associates and coagulates PEB molecules and these result in colloidal and larger size particle formation thus encapsulating and stabilizing the metals into particles and their aggregates. If, after acidic treatment, these PEB formations are dewatered (dried out), this irreversibly encapsulates the metals into coagulated particles and their aggregates. No metals removal is possible under any environmental or artificial conditions.

Note 6: *Encapsulated metals can be removed to some degree only by a number of special treatments: strong alkali (primarily sodium hydroxide) extraction at high temperature (around 200 C) and pressure (around 140 psi) and intensive mixing for one or more days resulting in some amount of PEB aggregate dissolution and supernatant formation. Supernatant is filtered and dried into solids then; solid phase is treated with strong acid for several hours resulting in PEB coagulation into solids and obtaining of the acidic supernatant, acidic supernatant filtration and drying into solids. Next steps include strong alkali extraction following by acidic treatment as it is described above. Finally, some amount of the metals can be extracted to supernatant - liquid phase or in other words converted to mobile forms.*

Note 7: *In general term encapsulation means harmful or dangerous substances filling into capsules which are made from inert material which insulates them from direct contact with environment. Uniform capsules and homogeneous surface is very important. The capsules*

breakage/surface disruption means toxic material release to the environment. This term "encapsulation" is applicable for PEB-metals complexes because the metals are located into volumetric molecules or inside PEB particles and aggregates being chemically bound and immobilized. There are no specific surfaces and there is no ability for any metal to release into the environment due to the metals immobility. It means metals stabilization.

The ratio between reversibly bound metals (ions exchange process) and irreversibly bound metals (complexes and chelates formation process) is a question of significant scientific interest and practical value. It was one object of our research in the CRADA (report is available as well as some other publications).

Two groups of metals were an object of research. First group: zinc Zn^{2+} , iron Fe^{3+} , copper Cu^{2+} and nickel Ni^{2+} . Second group: cadmium Cd^{2+} , lead Pb^{2+} , mercury Hg^{2+} and chromium Cr^{3+} . These were the metals salt solutions or in other words the metals cations and the corresponding anions (SO_4^{2-} ; NO_3^-). Ions exchange results in mobile metals (cations) content reduction without anions content reduction. PEB-metals complexes formation results in mobile metals (cations) content reduction and anions content reduction. The ratio between bound anions and cations indicates to dominating mechanisms in metals immobilization/stabilization.

Nickel and Zinc have zero ratio between bound anions and cations which means exclusively their immobilization through ions exchange. Iron indicates to 56% of bound anions to bound cations. Copper indicates to 36% bound anions to bound cations. This means both ion exchange and complexes formation.

Cadmium indicates to 12%; Mercury indicates to 34%; Chromium indicates to 43% and Lead indicates to 19% bound anions to bound cations. This means combined metals immobilization by ion exchange and complexes formation.

If the cations content exceeds the PEB ability to bind all of them (under high cations concentration and low PEB content) the cations compete for interaction with active groups and this results in the following hierarchy: $Pb > Cd > Hg > Zn > Ni$. This means that PEB immobilizes Lead first, then Cadmium, Mercury, Zinc and Nickel. So, some cations are more "native" to PEB than others. But if PEB concentration exceeds the metals content, there is no such type competition: PEB immobilizes all metals. The reaction which results in immobilization takes several ten seconds.

Note 8: *Heavy metals are toxic in their mobile, soluble forms. But one of them – Mercury – is very toxic in its metallic form – elemental Mercury which turns to extremely toxic vapor even at ambient temperatures.*

It is known, that elemental Mercury (Hg^0) has high affinity to activated carbon (that is why activated carbon is a common sorbent in exhaust gases purification from mercury at coal power stations) and organic carbon (that is why black soil with high humus content accumulates and contains much more Mercury than other soils).

PEB, being an organic carbon substance, has a high affinity to elemental Mercury forming stable covalent bonds $Hg-C-PEB$. These bonds are much stronger than Mercury-Activated Carbon bonds. This is a first and quick step in elemental Mercury detoxification. Second step is elemental Mercury (Hg^0) oxidation to Mercury cations (Hg^{2+}) on PEB functional groups (which lasts a longer time) and chlorides stimulates this process. Once formed, Mercury

cations rapidly interact with PEB through ions exchange, metal complexes and chelates formation as it is described above. In this case, Mercury is more strongly bound and immobilized/stabilized than in case of the original of Hg-C-PEB bonds.

The following are questions posed by clients to further clarify the information provided previously in this document. Some information is repeated to support the specific questions.

We state that the metals are bound, made immobile, converted into a non-toxic mineral metal, though the material containing the metals originally tests as a metal present at a particular level and we treat and test again, the metal is reduced 60, 80, 98%, whatever it may be, where did it go and why isn't the test showing it?

Response 1. We need to remember some well-known basics of fundamental chemistry and biology to explain how and why our products and technologies work. Metals, as chemical elements, exist in nature in different forms: natural forms like ores and "man-made" forms like salts for electro coating, dyes/paints, etc. Metals form ions—positively charged atoms or in other words cations. Metals naturally exist in nature: in water, soil, underground layers, etc. They naturally migrate in forms of soluble mineral salts – metals exist primarily in a form of ions - and organic-metals compounds: - metals cations are bound into complexes or chelates or just adsorbed on the organic colloids surfaces. Metals insoluble/immobile forms (metals ions that are tightly bound with other elements) cannot migrate thus they form different kinds of deposits. Metals ions, like most, if not all chemical elements in nature, can interact with other chemical elements and compounds – both mineral and organic, resulting in the different mobility of various metal containing substances and compound formations. These various metals exist in these substance and compounds as atoms with ion, covalent, complexes or chelate types of bonds with other elements. The strength of these bonds varies depending on many factors; meaning that metals atoms can be turned to ions and migrate out depending on physical, and chemical, and microbiological conditions. Naturally, there is not a problem with metals existence in nature (soil, water, etc.) however, the problem is in their mobility, which is caused by their ionic forms, concentrations, and ability to affect people through food chain, water, polluted air/dust, etc.

1.1 So, heavy metals are toxic if they are in mobile, primarily cationic (positively charged ions) form. For example, there are such common cations forming metals as Pb (lead), Cd (cadmium), Ni (nickel), Cu (copper), Zn (zinc), Co (cobalt), Cs (cesium), Sr (strontium) and others. Their toxicity depends on the rate of mobility and cations concentration: the higher the mobility and cations concentration, the higher their toxicity. They are toxic because they are being positively charged, they have a very high affinity to the negatively charged sites of human and plants cellular membranes (proteins), and their absorption by the cells results in: (a) metals cations penetration into cells through membranes where they disrupt native bio-chemical processes; for example, they affect DNA ability to replicate properly resulting in "mutagenesis", "cancerogenesis", and "teratogenesis". This is a dominating mechanism even when metal concentrations are relatively "low", but still high enough to affect the cells. (b) In cases of "highly" concentrated metals cations, once cations are adsorbed on the cells and bound with proteins they paralyze the cells ability to utilize nutrients and moisture absorption which results in rapid cellular death. The terms "low" and "high" are terms denoted by scientific research. In our situation associated with practical remediation, we are focused on the government

regulation for soil, water, waste, etc., depending on how these metals, being mobile (converted to cations), can affect environment, plants, animals, and humans. We operate with government approved metals content - something like "approved metals content" in soil, water, waste, etc. "Approved metals content," means some minimal mobile metals content, which is not dangerous for people.

Note: There are no completely insoluble substances and compounds in nature, even "insoluble" substances release a limited number of molecules, atoms, and ions to the level that is detectable by the modern chemical methods. That is why even insoluble compounds and substances with immobilized (immobile) metals release some amount of mobile metals – primarily ions. But these metals cations should not exceed the approved/permitted level.

"Approved metals content" varies depending on many factors. Even different states have different regulations in metals content. Now, the most common regulation is based upon the metals ability to be extracted from mineral and organic forms into cations (most toxic forms) by strong acids solution treatment. The primary method used by most states is the TCLP method. This method simulates heavy metals containing compounds which are in toxic forms and available through a variety of pathways into the human body through inhalation or ingestion into the stomach, which will interact with stomach's juice that is a hydrochloric acid solution at the pH around 2 and the metals conversion to metals cations associated with chlorides. On the converse to this, SPLP testing replicates similar environmental affects that would happen in everyday life and should be used in comparison or conjunction with TCLP to better demonstrate or measure how bond various metals are and how they will react in normal environmental conditions. TCLP is designed to rip all metals out and provide accurate numbers to the total levels needed to be managed where SPLP testing will more accurately demonstrate what impact and the true metals levels availability under normal weather conditions.

Note: strong acids solutions can chemically desorb (dissolve, mobilize) immobile metals due to their conversion into soluble salt, for example metals chlorides.

1.2. Some metals are toxic in anionic forms, for example hexavalent chromium and manganese (manganese oxidized form). Metalloid such as arsenic is very toxic in anionic and cationic forms.

1.3. Such metal as mercury is very toxic in its original form (due to its evaporation) and in cationic forms as well.

Metals contaminated materials are toxic because there are mobile forms of metals, or in other words they can release mobile metals that can reach people by migrating through soil, water, plants, and animals as a part of food chain or through dust inhalation and passing into the stomach. Once more, these metals exist in these contaminated materials not as metals alone (for example like metallic lead, cadmium, zinc, etc.). They exist in forms of soluble mineral salts (chlorides, sulfates, bicarbonates, nitrates, etc.); organic salts and some others that are being available to release ions. Our treatment of metals contaminated materials results in mobile metals (primarily metals cations) interaction with our substances chemically active functional groups that result in metals cations immobilization/stabilization/detoxification into organic-mineral insoluble compounds. It

happens due to ion exchange, complexes and chelate formation, or in other words due to chemical reactions. The metals are still in the material but are not available for migration thus they cannot reach people and do any harm to human health. And tests methods recognize how much metals were converted into mobile metals salts and were dangerous for people. The metal content reduction 60%, 80%, 98% means that respectively 60%, 80%, 98% metals were bound into stable organo-metals compounds and respectively 40%, 20%, and 2% metals cations still exist in mobile form, i.e. are extracted by the test method. Therefore, testing methods are designed to detect metals in forms that are toxic and hazardous, when materials are treated with our products, the form of the metals are altered into safe forms thus resulting in lower identification by accepted testing methods. We do nothing to blind the testing method. This commonly approved and accepted testing method cannot affect immobilized metals and turn them in the toxic forms/cations. It means that even under specifically created conditions (strong acids-hydrochloric and sulfuric at pH 1.5; intensive mixing for about 24 hours), metals are immobile and cannot affect people.

Note: some metals are more active than others. More active metals interact first and if there are not enough functional groups, other metals do not interact being still mobile. If there are enough functional groups, all metals interact and this results in all metals immobilization.

Note: other testing methods such as atomic absorption spectroscopy for atomized materials and the spectroscopy methods can detect all metals as chemical elements without any correlation with their chemical status: mobile/toxic or immobilized/nontoxic. But metals are a part of our common chemical elements environment on the Earth and only their toxic forms are objective of our concern/testing.

From a disposal standpoint, the ability to reduce the levels is a huge cost savings to everyone and significantly increases disposal options, from a liability standpoint, what type of event could release the metals from our treatment?

Response 2. The metals from our treatment are tightly bound/stabilized/immobilized into stable organic-metallic compounds. These are chemical and to some degree physical bonds. They are very stable under normal conditions: chemical environment, temperature, pressure, etc. **No known or possible natural event can release the metals from our treatment** in case of their deposition in disposal sites, landfill, etc. because of normal conditions. Only artificially created events can release some amount (not all) of metals.

For example, strong mineral acids solutions (hydrochloric, sulfuric, and nitric) can partially dissolve the minerals and release some metals in forms of chlorides, sulfates, nitrates. Strong alkali (sodium, potassium, and ammonium) solutions can partially dissolve organics and release some amount of metals. Numerous acidic and alkali treatment following by ultra-centrifugation; ultra-filtration, electro-dialysis allows most, but not all metals removal. Temperature above 600 C (1,200 F) can decompose organics resulting in some metals release. Intense (ultra-high level) UV radiation can result in organics partial decomposition and some metals release. All of these are impossible in case of treated material deposition under normal environmental conditions.

Normal weathering cannot change it significantly because of our product stable organic-mineral nature. For simplicity, it possible to say that our product's organic structure is extremely stable (insensitive) to strong acids, but relatively weak in case of strong alkali. At the same time our product's mineral part is insensitive (extremely stable) to strong alkali, but relatively weak to strong acids. However, the unique combination of both organic and mineral components combined together make metals treated by our products extremely stable.

Any scientific data on the binding potential and long-term validation that it remains bound?

Special research was conducted for three years on heavy metals contaminated soil. Control sites included the soil which was treated with hydrated lime for metals immobilization. Other sites were treated with our product to stabilize/immobilize metals. There was a huge metals release on the soil treated with hydrated lime, especially in the second year after treatment. No metals release was detected on the soil with our product that stabilized metals during three years of research.

Note: Our product contains stable organic and mineral fractions. Organic fraction is most sensitive to microbiological factors. Research of such kind organics behavior at normal soil conditions clearly indicated to around 5,000 year stability (according to 13C tests) in ancient soil (which was borrowed in geological media). Other tests indicated to around 800 year's organics stability in modern soil (top soil).

Special tests with our product treated material on metals stability have been performed and demonstrate no metals release after many years and additional computer modeling completed by independent testing agencies currently estimated the binding potential of materials treated with our products will have more than 300 years shelf life under intensive weathering, including microbiological processes.

This document was prepared with inputs from the entire 3 Tier Science Team to assist in the clarification of how our technology works and achieves many of the unique reactions. Our technology is based on science and though this document will shed light on some of the processes, it is not intended to clarify all possible questions. All readers are free to contact 3 Tier with specific questions or clarifications.