The fate of pollutants in soil

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ABSTRACT

Different disciplines must be urgently put together to understand environmentally related diseases, and to develop strategies capable of reducing the negative effects of pollution on human health. Within this framework, the importance of soils and their characteristics on human health is receiving a growing interest as the essentiality of soil for human life becomes increasingly clearer.

Understanding the transport and transformation of pollutants from the source of origin to the final receptors (environmental ecosystems and humans), through different soil typologies, is of paramount importance. On the basis of the duration, frequency and intensity of exposure, it is also important to evaluate which concentrations are necessary to produce biological alterations in living organisms, leading to the onset of a pathology.

One of the main objectives of the Environment and Health Inter-departmental Project, PIAS-CNR, was to highlight the close links between environmental matrices and human health. Within this framework, Working Group 1 (WG1) focused attention on the fate of contaminants in the environment, particularly on the soil ecosystem. The WG1 proposal aims to go beyond total diet studies and to understand mechanisms and processes by which contaminants enter the food chain and influence to various extents nutrition and the health of humans.

1. Introduction

The integration of different disciplines can help to overcome the compartmentalization of increasingly specialized scientific knowledge. This is urgently needed in order to understand environmentally related diseases, and to develop strategies capable of reducing the negative effects of pollution on human health. Within this framework, the importance of soils and their characteristics on human health has been growing in interest as the essentiality of soil for human life becomes increasingly clearer (38, 46, 75).

Soil functions, such as food production, are directly related to human health,. The action of filters on groundwater highlights the need to preserve soil given that its efficiency can be reduced by human activity and natural events (1, 108).

The fate of contaminants in soil is important in terms of evaluating their possible exposure to humans. Another significant element is the complexity of pathways determined by emission sources, interactions with soil surfaces, and changes over time in the chemical and biological conditions in the environment where the soil is located (Fig. 1).

2. State of the art and interactions between basic and applied research

Soil is defined as the top layer of the earth's crust and is made up of mineral particles, organic matter, water, air and living organisms. Soil is a multiphasic and

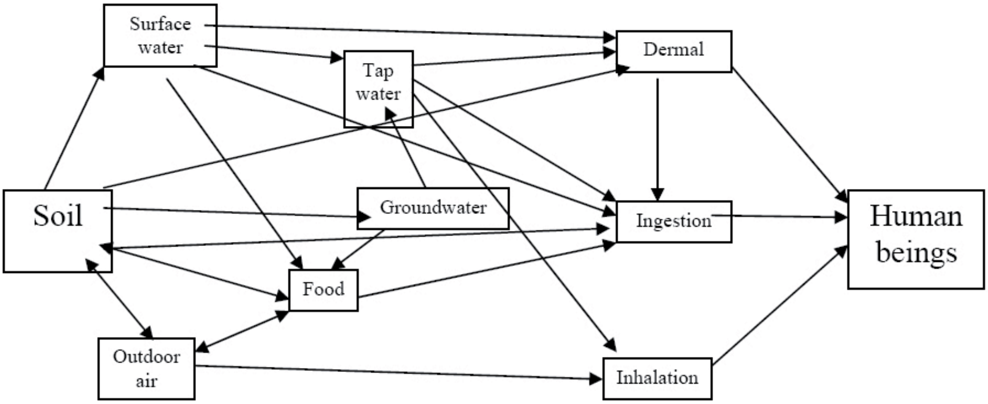

Figure 1. Soil – health relationships

extremely dynamic system, with numerous functions: it is the main producer of biomass and raw material, it supports biodiversity development (habitat, species, etc.), it provides the main source of carbon, and plays a fundamental role in human activities and in the survival of the ecosystem.

The formation and regeneration of soil are extremely slow and thus soil is considered as a non renewable source. Some soil degradation processes derive from anthropic activities (compaction, salinization, contamination, impermeabilization, decrease in organic matter, reduction in biodiversity), and natural phenomena (erosion, flooding, landslides). The resulting effects, however, can be aggravated by human activities, such as agricultural practices, industrial activities, tourism, urban and industrial development, and town and country planning (1). These processes can lead to a decrease in soil fertility, a loss of carbon and biodiversity, a reduced ability to retain water, an alteration in the gas and nutrient cycles, and a less efficient degradation of contaminants. Soil degradation has a direct effect on water and air quality and on climate changes. It can also influence human health and present a danger in terms of food safety (9, 33, 58).

Data analysis shows that soil degradation in Europe may cost 38 billion euros per year.

The Thematic Strategy of the European Union for soil protection (2006) proposes guidelines aimed at protecting soil and maintaining its ecological, economic, social and cultural role (30, 31). The Strategy is contained in the Plan of Environmental Action of the European Community, adopted July 2002 and valid until 2012. Its priorities include climate change, nature and biodiversity, the environment and health, natural resources, and waste (32). Co-ordinated action at a European level is necessary in terms of the consequences of soil degradation on other issues related to the environment or food safety. Before this directive was approved, soil had never been the focus of European protection measures, and soil protection was related only to regulations concerning environmental protection or other strategic fields, such as agriculture and rural development.

The Strategy is a legislative bill which permits the sustainable protection and use of soil, integrates soil protection within national and European politics, and raises public awareness. According to the directive, member states have to take measures to avoid soil contamination with dangerous substances and plan an inventory of contaminated sites.

When chemical concentrations present a risk for human health or the environment, the directive calls on member states to remediate the polluted lands, with the aim of removing, controlling, edging or reducing the pollutants. Similar strategies are also followed at an international level (25, 53, 74, 98, 99, 110, 132, 133, 134, 135, 139, 140, 141, 142, 143).

2.1 Soil and human health

Soil has always been vital to humans and fundamental to human health since it is the main resource for food production. The link between the continuously increasing world population and the ability of soil to sustain that growth was the topic of Thomas Maltus's 1798 essay. The maintenance of suitable nutritional food sources is an old problem that is still present today. Soil is not the only element that affects the food supply, but it is an extremely important resource needed in overcoming this complex issue. In developing countries characterized by a high rate of soil degradation, the lack of safety regarding an adequate dietary intake is a relevant problem. Worldwide food production and demand is likely to increase, making it crucial to manage and conserve soil. One of the the E.U objectives is to protect soils against erosion and pollution since long-term productivity is likely to be affected by soil degradation resulting in a relevant reduction in yields on agricultural land, not only in developing countries (77).

Soils may influence human health also in other different ways. Ingestion of soil may result in significant exposure to toxic substances. Children are the object of special interest, since soil adhered to fingers may be inadvertently swallowed by bringing the hands to the mouth, especially during outdoor activities (68).

One of the most common effects of soil ingestion is the alteration of the mineral content and nutrient balance in individuals. Ingested clays, due to the acidic environment of the stomach, release the elements contained within them through the mechanism of cation exchange (82).

Concerning the toxicity of the contaminants to which humans can be exposed through the ingestion of soil, lead is a major concern and focus of study. Children are subject to a greater risk because lead acts as a neurotoxin, with particularly serious effects on the development of the nervous system during childhood (96). Soil ingestion plays an important role in the risk assessment of contaminated sites, where also soil inhalation is considered of particular concern.

In the early 1980s studies revealed that most of the soil dust inhaled by humans is trapped and then swallowed, passing through the gastro-intestinal tract. However, a portion of this dust is trapped inside the lungs, where it can progressively lead to bronchitis, pneumoconiosis and cancer of the lungs. The reaction of the lungs to dust obviously depends on the kind and the amounts of the dust inhaled (137). Particles with a size comparable to those of clay and originating from wind erosion of the soil, once inhaled, can settle in the pulmonary alveoli, causing progressive inflammation in the lungs. Further damages arise following inhalation of particles coated with toxic substances and also of the biotic components of soil (20, 135). The fungus aspergillus present in the soil is the biggest killer along with the human AIDS virus, causing lung infections following immunosuppression (113). An infectious disease known as "desert fever" is caused by inhaling spores of the fungus *Coccidioides immitis*. In the U.S, it is estimated that each year between 50,000 and 100,000 people are affected by symptoms of Coccidioidomycosis (7). Tetanus is the most common desease to potentially affect people who come into contact with soil. This disease is due to a toxin produced by *Clostridium tetani* spores of anaerobic microorganisms. The bacteria, present in the surface layer of soils as well as in human and animal secretions, are especially abundant in cultivated and fertilized fields. Hookworm, characterized by multiple clinical manifestations including anemia, is a disease also caused by skin contact with the soil and whose hexogene agent is detectable in the nematodes *Ancylostoma duodenale* and *Necator americanus*. The survival of the hookworm larvae in the soil is favored in moist, sandy, crumbly environments and at temperatures between 24 and 32 °C. Infection can occur by oral ingestion of contaminated food and, presumably, direct ingestion of soil. The disease has a high incidence in the rural areas of the tropics, with higher occurrences in children (63).

Other diseases are ascribable to soil characteristics. Podoconiosis has been correlated with soils containing particles of colloidal size derived from wethering of basaltic rocks that are able to penetrate the epidermis intact (119).

Soil quality largely determines ground and surface water quality. In Bangladesh drinking water is contaminated with arsenic at concentrations of up to 1000 μg/l. The consumption of contaminated water led to the spread of disease and death, with typical epidermal lesions (105). Considering the sources of As and the mechanisms that result in groundwater pollution, it is possible that Fe hydroxides present in the sediments are reduced by the activity of microorganisms, favoring the release of As absorbed in groundwater (92).

When the soil ability to retain organic compounds by sorption processes is reduced, groundwater pollution by organics of industrial origin is a widespread problem. The same happens for pesticides that can penetrate into the soil through different routes, such as root systems, leaves and the decomposition of plant and animal tissue. Other pesiticides are directly applied to the soil and can be released from soil to surface water and groundwater (120).

Many of these compounds are considered endocrine disruptors and have severe health implications.

Further contamination derived from agriculture practices is the release of the NO_3^- anion from soil. This ion has a high solubility in aqueous environments, and being negatively charged is poorly absorbed by most soil surfaces. The excessive presence of nutrients causes algal bloom with serious consequences on the whole aquatic ecosystem. Toxins produced by rapidly growing cyanobacteria can cause numerous human disorders, including gastroenteritis, atypical pneumonia, allergic reactions, and liver diseases including cancer (12). Among the consequences of ingestion of nitrate ions is childhood methemoglobinemia, which severely damages the ability of hemoglobin to carry oxygen in the blood (73).

The use of antibiotics applied to agricultural crops for the control of plant diseases, and their addition to animal feed have given rise to several problems of immediate and practical importance. The inactivation of antibiotics in soil may be determined by: intrinsic chemical instability of the antibiotic molecule; adsorption on soil clay minerals and organic matter; microbiological degradation. Since antibiotics are a heterogeneous group of compounds, varying greatly in their chemical structure and reactivity, and soils are not homogeneous, no generalizations regarding the stability and biological effects of antibiotics in soil are possible. Although some antibiotics in soil are unstable chemically, and many are degraded microbiologically, it appears that several antibiotics persist in some soils for a time sufficient to produce harmful effect. Soil bacteria are considered to be a source of new resistance mechanisms to clinically used antibiotics. In Europe, the livestock industry consumes thousands of tons of antibiotics per year. The application of cattle manure to soil might be a relevant source of antibiotics. The bacteria populations resistant to antibiotics in soils are lower in unmanured soils than in feedlot soils.

In the U.S, the presence in groundwater of antibiotics such as tetracycline, added to feed to promote livestock growth, may present a possible means of determining antibiotic resistance in humans. The analysis of soil and ground water samples from reserves close to farms have shown that the bacteria are identical to those in the gastrointestinal tract of animals, and contain genes that are resistant to antibiotics (27). This study suggests that genes are transferred from bacteria of the gastrointestinal tract of cattle to other ecosystems. Since in the U.S. about 40% of the water used for civilian consumption comes from groundwater (and this value has been gradually increasing), the presence of antibiotics can lead to serious consequences for human health (136). In the EU, livestock consumes approximately 5000 tones of antibiotics each year. Though there is no set limit on the use of medicines in agriculture, veterinary authorities have ruled that any compound that can be accumulated at concentrations higher than 7.5 g per hectare must undergo environmental impact studies (114).

The deposition of feces in the soil from humans and animals may potentially contaminate fresh water sources with bacteria, protozoa and viruses (122). For example, *Escherichia coli* 0157 is a virulent pathogen that in humans gives rise to a broad spectrum of symptoms, including hemorrhagic colitis (83). Cattle are the main reservoir of the bacterium, which, once reaching the ground, remains there for several months. The most common causes of infection from *E. coli* 0157 are associated with the consumption of contaminated meat and dairy products, although infection in humans may also occur due to the contamination of soil and drinking water.

A noteworthy amount of metals has been released into the environment by anthropogenic activities, in particular by industrial processes and persist in the soil due to their non biodegradability. Heavy metal pollution is responsible for many negative consequences both for human health and the environment (17, 59, 61, 72). Most heavy metals are considered essential micronutrients and each of them requires an adequate daily intake. However trace elements are toxic if there are excessive amounts of them in the human body, and they have adverse physiological effects at relatively low concentrations. Soil ingestion represents a direct route for the elements to humans. The transfer of many elements from soil through the food chain is an important although indirect mean of exposure. Consequently, deficiencies, excesses or imbalances of inorganic elements from food sources may have important consequences. An inadequate intake of microelements is recognized as an important contributor to the global burden of disease through increased rates of illness and death from infectious diseases, and of disability such as mental impairment (16). An increase in the concentrations of microelements in soil derived from weathering processes of the parent rock material or by human activities such as industrialization, mining, agricultural practices, and urbanization, can cause an excessive release of elements in the food chain and can have implications on human health. The *itai-itai* syndrome is probably the best known example of metal contamination in the soil that has some implications for human health through the ingestion of contaminated food. It developed in Japan in the 1950s, and it is caused by food, especially rice, and drinking water contaminated by Cd (35, 97, 111).

As a consequence, all legislations concerning soil strictly regulate the soil cadmium content to avoid its accumulation in agricultural crops. However, Cd accumulation in plants is determined by the available fractions of metals in soil rather than their total content. Although the soils may contain high concentrations of metals or organic contaminants, factors such as pH, clay content, and organic matter impact on speciation, mobility and bioavailability of pollutants, influencing. the amount absorbed by animals and humans (131).

2.2 The fate of contaminants in soil

Soil contamination occurs through either point source or diffuse pollution; the main difference between the two types of contamination lies in how the contaminants are transferred to the soil. Point sources, such as manufacturers, landfills, incinerators, use soil as a support and are linked to the activities that necessarily transfer pollutants into the soil (64). Diffuse sources are associated with natural phenomena (long range transport, atmospheric deposition, sedimentation by surface water), with agricultural practices, with recycling and inadequate waste treatments. The most dangerous contaminants in soil are, in general, persistent organic pollutants (POPs) and inorganic pollutants, above all heavy metals. Persistent organic pollutants have an anthropic origin and are characterized by high lipoaffinity, semivolatility and resistance to degradation. In the case of heavy metals, that cannot be degraded or destroyed, the presence in the soil could be due to natural processes, for example the formation of soil, and to anthropogenic activities. Some are important essential elements (Cu, Fe, Mn, Zn, Co), if present in optimal concentration ranges, while others (Hg, Pb, Cd) are potentially toxic elements (19, 78, 80, 81, 84, 109, 130).

2.2.1 The nature and behavior of inorganic contaminants

Heavy metals are one of the numerous classes of substances that can reach critical levels in terms of human health, food safety, soil fertility and ecological risks (80, 126). Heavy metals are common contaminants in the soil and bioaccumulate, thus their concentration in the organism increases over time compared to the level measured in the environment. This is because the absorption rate is higher than the excretion rate in the organism (128).

The distribution of heavy metals between the solid phase and the soil solution is considered to be the key factor when assessing the environmental consequences of the accumulation of metals in the soil (2, 69).

A physical and chemical analysis along with an analysis of the soil profile is essential for assessing the soil as a barrier against inorganic contaminants, particularly heavy metals (121). The retention of heavy metals in the solid phase of the soil is dependent primarily on the pH, and is linked to clay minerals, humic substances, iron oxides and hydroxides, and manganese found in

the soil, which all control the attenuation effect even on anionic forms (116).
The retention and release process of heavy metals includes precipitation and decomposition, ionic exchange, and adsorption and desorption.
The precipitation/release reactions may involve discrete solid phases or solid phases, which are absorbed onto the soil surface. The ion-exchange reactions derive from an exchange between an ionic species in the soil solution and an ionic species retained in sites with permanent charge on the soil surface. The absorption and desorption processes can affect all ionic or molecular species and generally concern absorbent sites with a pH-dependent charge. These surfaces are iron, aluminum and manganese oxides and hydroxides, clay minerals and humic substances.

pH

pH is the most important parameter governing concentrations of metals in soil solutions that regulate precipitation–dissolution phenomena. Metal solubility tends to decrease at a higher pH. In alkaline conditions the precipitation of solid phases diminishes the concentration of metal ions in solutions and the reverse happens with a lower pH. pH values also regulate specific adsorption and complexation processes. The sorption of metals is often directly proportional to soil pH due to the competition of H^+ (and Al^{3+}) ions for adsorption sites, however this competition may be reduced by specific adsorption. Metal hydrolysis at higher pH values also promotes the adsorption of the resulting metal hydroxo complexes, which beyond a threshold pH level (which is specific for each metal) drastically reduce the concentration of metal ions in the soil solution. At low pH levels, on the other hand, sorption processes are reduced due to the acid catalysed dissolution of oxides and their sorption sites, whereas the complexation by organic matter tends to decrease with increasing acidity.

Clay content

Ion exchange and specific adsorption are the mechanisms by which clay minerals adsorb metal ions. This is done through the adsorption of hydroxyl ions followed by the attachment of the metal ion to the clay by linking to the adsorbed hydroxyl ions or directly to sites created by proton removal. Highly selective sorption occurs at the mineral edges. However notable differences exist among clay minerals in their ability to retain heavy metals which are more strongly adsorbed by kaolinite than montmorillonite. This is probably due to a higher amount of weakly acidic edge sites on kaolinite surfaces. In expandable clays (vermiculite and smectite) the sorption processes essentially involve the inter-layer spaces, and are greater than in non-expandable clays such as kaolinite. The importance of clay minerals, and of soil texture in determining the distribution of heavy metals between the solid and the liquid phases of soil has direct consequences on the metal bioavailability of plants. For the same total concentration it is well known that heavy metals are more soluble and plant available in sandy soil than in clay soil.

Organic matter content

The organic matter content of soils is often small compared to clay. However, the organic fraction has a great influence on metal mobility and bioavailability due to the tendency of metals to bind with humic compounds in both the solid and solution phases in soil. The formation of soluble complexes with organic matter, in particular the fulvic fraction, is responsible for increasing the metal content of soil solutions. However higher molecular weight humic acids can greatly reduce heavy metal bioavailability due to the strength of the linkages. Both complexation and adsorption mechanisms are involved in the linking of metals by organic matter thus including inner sphere reactions and ion exchange. Negatively-charged functional groups (phenol, carboxyl, amino groups etc.) are essential in metals retained by organic matter. The increase in these functional groups during humification produces an increase in the stability of metal organic complexes, which also show a greater stability at higher pH values.

Cation exchange capacity
The density of negative charges on the surfaces of soil colloids defines the CEC of soil. This capacity is governed by the type of clay and amount of organic colloids present in the soil. Montmorillonitic type clays have a higher net electrical charge than kaolinitic type clays; consequently, they have a higher cation exchange capacity. Soils containing a high percentage of organic matter also tend to have high cation exchange capacities. The surface negative charges may be pH dependent or permanent, and to maintain electroneutrality they are reversibly balanced by equal amounts of cations from the soil solution. Weak electrostatic bonds link cations to soil surfaces, and heavy metals can easily substitute alkaline cations on these surfaces by exchange reactions. Moreover, specific adsorption promotes the retention of heavy metals, also by partially covalent bonds, although major alkaline cations are present in soil solutions at much greater concentrations.

Redox potential
Reduction-oxidation reactions in soils are controlled by the aqueous free electron activity pE often expressed as Eh redox potential. High levels of Eh are encountered in dry, well aerated soils, while soils with a high content of organic matter or subject to waterlogging tend to have low Eh values. Low Eh values generally promote the solubility of heavy metals. This can be ascribed to the dissolution of Fe–Mn oxyhydroxides under reducing conditions resulting in the release of adsorbed metals. However under anaerobic conditions, the solubility of metals could decrease when sulphides are formed from sulphates. Differences in individual metal behaviour and soil characteristics result in conflicting reports regarding the effects of redox conditions on metal solubility.

Iron and manganese oxides
Hydrous Fe and Mn oxides, are particularly effective in influencing metal solubility in relatively oxidising conditions. They are important in reducing metal concentrations in soil solution by both specific adsorption reactions and precipitation. Although Mn oxides are typically less abundant in soils than Fe oxides, they are particularly involved in sorption reactions with heavy metals. Mn oxides also adsorb heavy metals more strongly, thus reducing their mobility. This action is particularly important in contaminated soils. Specific adsorption of metals by hydrous oxides follows the preferential order: Pb > Cu >> Zn > Cd.

Other factors
There are a number of other factors which may affect the solubility of metals in soils. Temperature, which influences the decomposition of organic matter, can modify the mobilisation of organo-metal complexes and consequently plant uptake. An increase in the ionic strength of soil

solutions reduces the sorption of heavy metals by soil surfaces due to the increased competition from alkaline metals. Similar effects also derive from the simultaneous presence in soil solutions of many heavy metals which compete for the same sorption sites. This results in an increase in mobility in contaminated soils due to the saturation of adsorption sites. The living phase of soil is also of great importance in determining metal solubility, which is dependent to some extent both on microbial and root activity. In the rhizosphere, plants can increase metal mobility by increasing their solubility. This happens following the release in the exudates both of protons which increase the acidity, and organic substances which act as complexing agents. Microbial biomass may promote the removal of heavy metals from soil solutions by precipitation as sulphides and by sorption processes on new available surfaces characterized by organic functional groups.

2.2.2 Organic pollutants

Among the many organic compounds present in soil, the most dangerous are the "persistent organic pollutants" that derive, in general, from anthropic activity, are extremely persistent in the environment and are transported for long distances (5, 28, 55, 66, 71). In specific environmental conditions they bioaccumulate and biomagnify, reaching considerable concentrations that represent a threat for human health and ecosystems. Of the twelve groups of persistent organic pollutants, the following three are acknowledged internationally: polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

PCBs are high hydrophobic extremely stable compounds and have very good dielectric and thermostability properties; these characteristics led to the diffusion of PCB for industrial and civil use (8). After accidental ingestion or due to their presence in food compounds, PCBs are absorbed through the gastrointestinal tract, and then accumulate in body fats as a consequence of their hydrophobicity (85). The International Agency for Research on Cancer (IARC) has classified PCBs as potential carcinogenic agents for humans: experimental tests suggest, in fact, that these compounds may increase the risk of skin, liver and brain cancer (24). In order to protect human health and the preservation of the environment, the European Community banned the commercial use of PCBs in 1990. However, these persistent compounds are still present both in natural soils, owing to long-distance transport, and in soils that have been contaminated by specific industrial activities (13).

PCDDs and PCDFs, which are generally known as "dioxins" (118), are the undesired by-products of chemical and combustion processes and are also produced from natural events, such as accidental fires and volcanic eruptions. The dioxins are a group of 210 chlorine-containing chemicals, 17 of which have a toxicological interest owing to their carcinogenic potential and their effects on reproductive, endocrine and immune systems (48). Owing to their high persistence in the environment, they remain in soil, which become pollutant reservoirs (117). In humans, the main route of exposure to dioxins is through food, which represents 90% of the total exposure (51, 87).

EDCs. Over the last few years there has been an increasing interest in identifying the long-term damage to reproduction and development; xenobiotics with potential endocrine activities or endocrine disrupter chemicals (EDCs) have been identified as the main possible risk factors (47).

Endocrine disrupters are a heterogeneous group of persistent organic and inorganic pollutants including dioxins, PCBs, pesticides, and industrial compounds. They are characterized by their potential to affect the correct functions of the endocrine system, especially the homeostasis of sexual and thyroid hormones (10, 29, 125). These molecules may enter the soil environment by agricultural practices or industrial waste disposal. The risks derived from EDCs are determined by the distribution of these compounds among the soil phases. Depending on the chemical properties of the molecules, EDCs can be either strongly retained by solid soil phases, or leached to deeper layers. Their mobility is largely determined by adsorption – desorption processes on solid soil phases.

A probable role of endocrine disrupters is attributable to polybrominated byphenyls (PBDEs), a class of manufactured chemicals structurally similar to PCBs, which were used in the past as flame retardants (15, 40). Even though most PBDEs were banned within the European Union in 2006, studies have revealed that PBDE levels have increased both in the environment and in human tissues and body fluids (34, 37, 50, 60).

Pesticides are a class of compounds used to kill harmful organisms, especially in agriculture. However many are also toxic for other organisms, including humans (93). The presence and bioavailability of pesticides in soil can adversely impact soil quality with related consequences on water and air quality. Soil characteristics regulate the processes that affect the behavior of pesticides such as adsorption, degradation, volatilization adsorption by crops. Pesticide adsorption to soil depends on both the chemical properties of the pesticide and properties of the soil, in particular organic matter. Organochlorinated pesticides have been used for many decades and one of their main features is their high persistence in soil and transfer into the food chain, with the consequence of well known toxic effects in biota (67).

Behavior of organic contaminants

Organic molecules in soil are a carbon source for microorganisms. Therefore, the conditions that influence the breakdown of organics by microflora should be considered. Microflora are not always able to attack organic molecules and digest them completely, but often only partially break them down. This results in compounds that are even more toxic than the initial ones.

The intrinsic toxicity and health risks following the ingestion of organic compounds are well known, both natural compounds and those deriving from productive processes. On the other hand, there is less information about the potential contamination, caused by organic compounds present in the soil, on the food chain (plants-animals-humans). Organic compounds should be evaluated in terms of their chemical properties and their relative absorption potential by plants, but also in terms of the influence that the soil has on them. In fact, these compounds can be volatized, absorbed and therefore immobilized, or transported along the soil profile even to underground water.

The most important chemical properties of organic molecules are those dealing with their absorption in the food chain: the distribution coefficient (octanol/water (Kow), the Henry constant, solubility, half-life, and the bioconcentration factor (BCF).

The behavior of an organic contaminant in the soil depends on the interactions that are established with the solid, liquid and gas phases of the soil, and with the living phase. These relations give rise to the major phenomena that rule the fate of the organic contaminants concerning adsorption, biotic and abiotic decay, leaching and volatilization.

Adsorption and desorption

The adsorption processes of organic compounds on the active surfaces of the soil are particularly important because they delay mobilization and leaching of organic contaminants.

The distribution of the contaminants between the liquid and solid phase of the soil can be synthetically described by the distribution coefficient Kd, which in turn can be expressed as a function of organic carbon (Koc) and of Kow. The compounds that have high levels of Kow and low solubility will be mostly retained by the soil surfaces and be less available to environmental processes.

Biodegradation

Biodegradation is the most important mechanism for the removal of organic compounds in the soil. Degradation by the microbial flora can increase the solubility and therefore the availability of recalcitrant compounds for microorganisms in the soil. The chemical characteristics of each specific compound affect the time required for biodegradation. Various parameters have been identified that could be correlated with the degradation period. For example, the half-life of polycyclic aromatic hydrocarbons, PCBs and dioxins are all related to the Kow. This coefficient is also related to the leaching process and to the persistence of contaminants in the soil. In fact, compounds characterized by a log Kow > 4.0 rarely mobilize. Therefore the same compounds mentioned above, as well as several organochlorinated pesticides are very persistent and have a very low leaching potential. Monocyclic aromatic hydrocarbons, some chlorobenzenes, short chain aliphatic compounds and phenols, on the other hand, degrade rapidly and are more easily leached from the soil.

Photolysis, hydrolysis and oxidation (abiotic degradation) also contribute to the disappearance of some organic compounds. These reactions mostly affect compounds with simple molecular structures, such as phenols and some polycyclic aromatic hydrocarbons (PAHs) with less than four benzene rings. Volatilization also affects volatile substances, which are generally characterized by a reduced molecular complexity.

2.2.3 Soil - animal transfer

Depending on the grazing practice, the season and the diet, both organic and inorganic compounds present in soil with a high level of contamination are potentially transferable to animals. In fact, contaminated soil is usually ingested directly during grazing. The average amount of soil ingested by most cattle is around 6% of total removal (d.w, dry weight), i.e. for a typical daily bovine consumption of 15 kg d.w. it can reach 0.9 kg of contaminated soil per day. Assuming that organic compounds in polluted soils are present in concentrations from 0.1 to 10 mg/kg, the amount ingested can vary from 30 to 3000 mg/year

Bioconcentration processes are particularly important for persistent and non-polar compounds (low solubility and with high Kow). The BFC bioconcentration factors related to diet, however, are difficult to quantify experimentally. Nonetheless, some models based on the daily intake of organic compounds can provide an estimate of their possible presence in meat or milk.

When ingested these compounds can pass through the gastrointestinal membrane,

enter the blood or lymphatic system or into some organs in relation to the lipid content and, depending on the compound (PCBs, dioxins, hexachlorobenzene), may have long half-lives.
Other compounds such as polycyclic aromatic hydrocarbons are not particularly absorbed, but can be partially degraded with the consequent formation of very dangerous intermediate products.
Among foodstuffs, milk is especially sensitive to organic compounds which undergo considerable changes in the concentration of organic compounds even in response to short term changes.

2.2.4. Concluding remarks

More research is needed in order to evaluate if the pollutant levels in the environment threaten human health, considering the most susceptible situations (proximity to contamination sources such as landfills or contaminated sites) and the most exposed people, e.g. owing to work activities or diet (6, 23, 54, 57). One very important aspect is an understanding of the transport and transformation of pollutants from the source of origin to the final receptors (environmental ecosystems and humans), through the different soil typologies (64, 103, 106, 129)). On the basis of the duration, frequency and intensity of exposure, it is also important to evaluate the concentrations necessary to produce biological alterations in living organisms, until the onset of a pathology.
Diet represents the principal means through which not only chemicals, but also micro-organisms and mycotoxins, reach humans (52). Research is needed on the bioavailability mechanisms of organic and inorganic pollutants in soil, and the survival and persistence quantification of pathogen agents in the environment. The detection of microbic interactions is also essential, together with further knowledge of emerging pathologies and molecular toxicology. Finally, studies need to be performed on the long-term effects following chronic exposure to low levels of individual or mixed chemicals, as well as the consequences of exposure to high concentrations of natural elements.

3. Pilot Study: Influence of soil characteristics on the mobility of contaminants in the industrial area of Gela (Sicily)

3.1 Soil characteristics of the Gela site

Gela is a town in Sicily located in an area of important industrial activity, which has caused over the course of time significant contamination of the environment. In fact, it is so polluted that the Italian government has designated it as an area (in Italian known as “Site of National Interest: SIN”) that is subject to specific regulations in terms of remediation. The Gela site lends itself to a new interpretation of pollution that is no longer confined to a contaminated site, but which has instead spread to a wider environmental area. The *Institute of the Ecosystem Studies* (*CNR-ISE*, Pisa) have tried to highlight how important soil characteristics are in defining the contamination pathways of pollutants, and how complicated it is to establish general relations since each type of soil has specific characteristics that differentiates it from other types.
Given the complexity of the matrix, which can contain many organic and inorganic contaminants, the definition of a potential hazard based on soil characteristics, is limited. It is only thorough a characterization of the soil of the industrial area of Gela affected by pollution that it would it be possible to give a more accurate response. However,

given the amount of data produced during the characterization phase of the site, we planned to assess the characteristics of the soils derived from analysis certificates, based on the analytes determined. We also wanted to evaluate which soil parameters were missing, which are useful to detect contamination pathways.

As mentioned previously, the potential hazards of a contaminant present in the soil and its risks to human health, particularly through the exposure pathways from the soil through the food chain and finally to man, can be better defined if we know the chemical and physical properties of the soils in which contaminants are present (1, 2, 22).

We examined the documentation collected at the Italian Ministry of the Environment on Gela site to check whether, in addition to the characteristic parameters of contamination (pollutants concentration), there were also parameters describing the characteristics of the soils. This was because these factors would help to predict the environmental mobility and potential bioavailability of the contaminants present.

The parameters that were observed and that are most frequently reported are pH, cation exchange capacity (CEC) and organic carbon (C). These quantities are fundamental to understand what types of soil are in the areas concerned and how they interact with the contaminants in the soil. These data are shown in Figures 2, 3 and 4. However, there are no data on texture, which is of paramount importance. Such data could possibly be obtained, even if only partially, from the geological description of any probing that might have been done.

Given the significant amounts of data on the characterization of soils within the various industrial areas, some conclusions can be drawn with regard to heavy metals. However, based on the data reported on the certificates, it is not possible to make concrete hypotheses regarding organic contaminants. Among the parameters identified, pH is particularly constant and depends on the nature of the mineralogical substrate from which the soil originates. In fact, it is the most important parameter that governs the concentration of inorganic elements in soil solutions. In the soils from the Gela area there should both be a limited mobility of heavy metals, which move like ions with positive charges (Cd, Zn, Cu, Pb), and a limited bioavailability of such metals. The same should hold true for mercury, however the probable presence of high concentrations of chloride ions can greatly facilitate the mobilization of the element and its diffusion in the environment. Metals that move like ions on the other hand, with a negative charge such as As, may be more easily mobilized, and could become a significant problem in the whole area. The other parameter reported in the characterization analysis of soil is the cation exchange capacity. This quantity expresses the charge density on the surfaces of soil colloids. It varied considerably from one industry to another which meant it was impossible to identify a uniform retention capacity of metals in different parts of the Gela area under investigation. The importance of pH and CSC is not as significant in organic compounds as it is in inorganic contaminants. These parameters have a limited influence on the mobility of nonionic organic compounds, which are much more influenced by the content of organic matter. Organic matter in the soils of this area appears to be quite low although there are considerable differences between one area and another. Variability in the values of organic matter and the lack of soil texture characteristics are of primary importance for understanding the behavior of organic contaminants. This highlights the need to integrate data from the characterization of the site with the features of the soils, which could be partly drawn from land use maps at a provincial or regional scale. The characterization of environmental matrixes can be a key issue for population exposure estimates, integrated with data on health and epidemiology. As far as soil is concerned, in addition to the commonly used ways of evaluating a contaminated site, such as skin contact and direct ingestion, it is also necessary to take into account the characteristics of this environmental matrix affected by the contamination. Soil properties determine the movement of pollutants and their passage into the food chain (1). This leads to an understanding of low-dosage effects, which are prolonged over time and which are often forgotten in decontamination strategies dictated by the need to solve immediate and acute problems resulting from pollution.

By understanding the specific soil characteristics of the area, if possible along with a related food analysis, uncertainty in the exposure calculations may be reduced and a relationship can be defined between the sources and targets of the contamination.

In order to evaluate the capacity of the soil to interact with different types of pollutants, it is necessary to consider this environmental matrix as a three-phase system. In general, the solid phase constitutes 50% of the soil, while the other half is made up of a porous space which, in a good quality soil, contains half water and half air. The solid phase, the degradation of the parent rock, contains organic materials (humic substances) that are concentrated in the upper layers. It also contains inorganic materials which at a certain depth become the exclusive constituents of this phase. The liquid phase is made up of water that forms a “soil solution”. This solution contains dissolved substances and can dissolve other substances from the solid phase. The soil solution reaches the roots and gets into pores. This is the principle means of transport of all of the substances, including the pollutants. The gaseous phase of the soil is made up of air, which on the surface layer is richer in carbon dioxide because of the high quantities of organic material.

The flow of air into and out of the soil is essential for plant growth and for the decomposition processes of animal and plant residues, as well as all materials of an organic nature.

Mercury is particularly important in the Gela site. Mercury, like other metals in the soil, may be present in a dissolved form as a free ion, or absorbed non-specifically by weak electrostatic bonds, specifically absorbed by covalent bonds, made more complex by organic matter, or precipitated in its solid phase in the form of carbonate, hydroxide or sulfide (18). Depending also on redox conditions, mercury can exist in three valence states, Hg^0, Hg and Hg^{2+}. Its bivalent form is generally highly reactive with dissolved ligands, and is highly soluble in water. It very often forms complexes with Cl^-, OH^-, S^{2-} and with sulfur-containing functional groups of organic compounds and NH_3.

Mercury also forms complexes of moderate stability with Br^- and I^- and some nitrogenous R-NH_2-type binding agents. The factors that control the speciation of the metal in solutions are pH, ionic strength, redox potential, a concentration of dissolved organic matter (DOM), and dissolved ions such as oxygen and sulfides.

The maximum solubility of mercury occurs in an oxygenated environment (Eh 350-400 mV) which is the typical condition found in soil. The principle forms that are found in soil are $Hg(OH)_2$ and $HgCl_2$. With these ions, mercury can form soluble complexes that are environmentally significant because they are very mobile. On the other hand, in anoxic environments these ions form stable and insoluble sulfides.

Methylmercury, CH_3Hg^+, and dimethylmercury, $(CH_3)_2Hg$, are also formed in the soil (49, 90) but they constitute on average less than 2% of the mercury present in the soil. Even at low concentrations, these compounds can cause serious bioaccumulation problems (101). CH_3Hg^+ is synthesized by microbe activity (bacteria and fungi) both aerobically and anaerobically. It is soluble in water and forms different compounds such as CH_3HgCl, CH_3HgOH and CH_3HgSH. The anion that binds itself is particularly important because it determines the biological uptake. The speciation of the CH_3Hg^+ ion is similar to Hg^{2+} and therefore the parameters that influence it are the same: pH, DOM and ionic strength.

Mercury is mobilized in the soil through the formation of soluble inorganic compounds which include $HgCl_2$ and $Hg(OH)_2$. The degree of mobility of these complexes depends on the type of charge and on the chemical and physical characteristics of the soils in the area. The presence of chloride ions makes the metal highly mobile for the formation of very soluble complexes.

In the presence of high amounts of organic substances, a process that is equally important is the formation of organic complexes of bivalent mercury due to the high affinity of the Hg(II) ion and of its inorganic compounds for the functional groups containing sulfur. A part of bivalent mercury can be complexed by soluble humic substances, such as fulvic acids, and therefore may be present in the liquid phase of the soil. Mercury loss due to ground runoff is still very small compared to the total percentage, so that in contaminated soils like those in the Gela area the metal can be expected to be released for a very long period, thus affecting human health for many years.

While historically mercury has been the element of greatest concern in the area of Gela, the soil characteristics are more favorable to provide conditions for a great mobility and bioavailability of arsenic. Like other metals arsenic toxicity depends on the chemical form of the element, organic compounds being much less toxic than inorganic ones. The main forms of arsenic in soil are arsenate and arsenite which are highly toxic, because their molecular similitude to phosphate can interfere with the functions of many proteins. USEPA defined arsenic as a human carcinogenic contaminant.

Soil arsenic may influence human health by soil dust respiration, soil ingestion and consumption of contaminated water (3). Moreover arsenic may enter the food chain via crops and vegetables grown in polluted soil (62).

The total content of arsenic in soil is not a reliable indicator of the potential hazards for health and environment. Its mobility and bioavailability are largely determined by soil characteristics (104). The retention of arsenic in the solid phase depends on soil pH, mineralogical composition and competing ions in soil solution. In aerobic soils, sorption on metal oxides is the main process that regulates arsenic bioavailability. Arsenate is linked to amorphous Fe and Al oxides, by the formation of inner sphere surface complexes, while arsenite forms inner sphere and outer sphere complexes, the latter specifically with Al oxides.

In the soil from the area of Gela, the trend of arsenic mobility is inverse to that of mercury. Soil pH determines the negative potential of mineral surfaces, which increases with increasing pH. The net effect is a decrease in sorption processes in the solid phase of soil. The transport of arsenic in soil is controlled by sorption/release processes and the alkaline conditions of these soils together with the oxidation – reduction potential, promoting an increase in the mobility of arsenate ions. Movement of the contaminant is determined by a pore space diffusion coupled with a sorption on solid phases which can be described by a Freundlich type equation. The solubility of arsenic can be described by a distribution coefficient Kd of the divalent arsenate ion which is directly dependent on soil pH according to the equation

$$\mathrm{Log}_{10}\ K_d = \log_{10}\ (\mathrm{As\ soluble}/H_2AsO^-_4) = a + b\ \mathrm{pH}$$

Where K_d is the solid solution distribution coefficient, for the arsenate ions As_{sol} the amount released in solution and $H_2AsO^-_4$ the free ion activity (127). From this equation potential bioavailable arsenic substantially increases with increasing pH. In the environmental conditions of soils in the area of Gela there is a high probability of the existence of soluble arsenic forms. The soil characteristics, would seem to indicate that the hazards deriving from this element could be even higher than those deriving from mercury contamination.
Environmental issues regarding metals such as mercury, arsenic (11, 14, 70, 91) are strictly linked to soil characteristics in that immobilization or potential bioavailability is regulated by parameters that are specific to the soil (pH, clay content, organic matter, and cation exchange capacity). These determine the chemical and physical conditions that may give rise to precipitation or solubilization resulting in an increase in bioavailability and/or leaching, with a danger of polluting the aquifers.

3.2 Contamination pathways of organic compounds

In an area with a high degree of pollution such as the Gela site, the main pathways of contamination affecting the soil are, in addition to skin contact and direct ingestion of soil, absorption by roots, transfer to the edible part of plants, and direct soil ingestion by animals during grazing.
The absorption by plants of organic compounds present in soils is influenced by the physical and chemical properties of the compound, by the type of soil and by the characteristics of the plant. It can occur both by radical absorption and by subsequent translocation in the aerial part, both by leaf absorption of volatile compounds and contaminated dust.
These issues vary in importance depending on the compound in question. Hydrophobic substances (PCB) can be absorbed on the root surface and remain bound to the lipid of membranes. This can create serious problems in some species such as carrots, which have an ectoderm rich in lipids. Plant absorption is a complex phenomenon based either on an active process specific to each compound, or a passive process in which organic contaminants are transported by the transpiration water of the plant.
There are several indicators for predicting the transferability of organic compounds from the soil to the plant. For example, compounds with a log Kow between 1 and 2 are those most likely to be moved by the aerial part of the plants. Substances with a half-life of less than 10 days will tend to disappear from the soil before being absorbed by the plants, while the most persistent ones can get into the plant

nutrition processes. The most volatile compounds with Henry's constant > of 10^{-4} tend to evaporate from the soil and given that they are not absorbed by the roots they may contaminate the plants through the leaves by volatilization. Of course, this is a very schematic approach that can be used for an initial screening of polluted soils in order to understand what the immediate dangers are.

The passage of an organic contaminant from the soil to the food chain can be described as a series of consecutive partition reactions between the solid and liquid phase of the soil, between the soil solution and the roots, and between the roots and the aerial part of the plant. This series of reactions is influenced by the characteristics of organic compounds, in particular by the partition coefficient of octanol/water, so that compounds with a low Kow value can be moved more easily into the aerial part of the plants. On the other hand, substances with a high Kow value (PAHs, PCBs, PCDD/F) are adsorbed by the soil and, if partially uptaken by the plants, they remain in the root system (2). Generally, these compounds are not absorbed, but there can be an accumulation of some compounds in root crops (confined to the outer parts of the roots that are removed before consumption).

Some of the more volatile compounds may enter the leaves, especially through the stomata, by atmospheric deposition or by absorption of the molecule in its vapor state. For semi-volatile compounds with a high Kow, translocation from the root system may be minimal, so the absorption in the vapor state can become an important source of leaf contamination. Compounds with a high lipophilic nature and high volatility may be present with significant concentrations in the leaves.

Inside the plant, some substances may be metabolized in a short period, others (PAHs, PCBs) much less, though they may be partially degraded at specific sites. For example, some nitrobenzene compounds are degraded in the roots, while some aromatic chlorinated compounds are metabolized only in the leaves.

Metabolism takes place depending on the structure of the chemical contaminant and the type of plant. For example, when degradation increases, it decreases the number of chlorine atoms, and the process is often only partial with the formation of intermediates.

The amount of halogenated organic compounds that can be absorbed by the plant, including hexachlorobenzene, a contaminant of interest in the Gela area, depends on water solubility, the concentration and organic matter content in the soil. The immediate risks stem from whether the plant is able to metabolize or eliminate the compound before being harvested, and whether the compound is transferred to the edible part.

Hexachlorobenzene can result from various industrial processes. It is very stable and not particularly reactive, since it is involved in the adsorption phenomena at the soil surface, which influence the volatilization and leaching processes as well as its preponderance to biological and chemical degradation or uptake by plants.

Since it is a non-ionic compound, it is subject to the adsorption process involving Van der Vaals forces, and it is closely linked to the content of organic matter, particularly in soils with a low clay content. Unlike other chlorobenzenes it has a log Kow > 5.3 and is therefore difficult to assimilate by the plant since it is substantially immobilized by adsorption processes in the soil.

The molecular structure is such that the leaching process should be quite limited. However, depending on the characteristics of the soil texture, the compound may be found in groundwater, transported through the larger pores in the soils, or in soils that have a tendency to form deep shrinkage structures.

The chemical stability of hexachlorobenzene makes it particularly persistent in soil and resistant to biodegradation, with a half-life of more than 1500 days. This compound has a remarkable permanency in the atmosphere with the possible formation of hydroxyl radicals and a half-life of two years, which could reach the soil as a result of precipitation and atmospheric deposition.

The principal biodegradation mechanism is oxidation, which leads to the formation of hydroxylated aromatic compounds, followed by the breaking of the benzene ring.

Hexachlorobenzene tends to accumulate in the roots of plants and remains bound to lipids of the membranes and the cell walls with less possibility of translocation due to its low solubility.

The potential toxicity of hexachlorobenzene for animals is largely linked to the risk of direct ingestion of soil by animals during grazing or through fodder feeding. Hexachlorobenzene is characterized by a high volatility that can be an important pollution pathway, through leaf absorption of fodder and consequently by animals.

3.3 Conclusions

Soil is a complex system that has allowed life on earth to exist and facilitated the birth of agriculture. In addition to being the most important source of essential nutrients, it is also a source of pollutants that reach humans through the food chain and diet (1). Given that soil quality is vitally important for our health, it is surprising that this issue has been studied so little by the scientific community (86). This probably stems from the fact that identifying and understanding the mechanisms linking soil quality and health, through the intake of agricultural products or processed foods, requires detailed and multidisciplinary expertise which is difficult to coordinate (42, 43, 44, 45, 46).

An innovative solution is to overcome the compartmentalization of environmental aspects and consider a continuum that goes from the presence of a substance in the soil, to its transfer into the food chain with the consequent health effects (94). The main transfer pathways of substances from soil to humans have been studied almost exclusively within contaminated sites. It is assumed in rather simplistic terms, that there is a direct correlation between the concentration in the soil of a given element (or substance) and its absorption by man (39). However, what really needs to be investigated is how, in a broader context the transfer of contaminants from soil to humans follows quite complex pathways (76). These pathways are determined by the chemical and physical nature of soil (115) characterized by physical, chemical and biological equilibriums in a multiphase system that is thermodynamically open.

4. CNR Specific expertise: qualified teams and external collaborations

4.1 CNR Institutes

The *Institute of the Ecosystem Studies* of Pisa (CNR-ISE, Pisa) is involved in the study of the quality of soil and of shallow and ground waters. This is because they play an essential role in life cycles, ecosystems and our quality of life.

The study of soil quality related to human health has not been studied much by the scientific community. The understanding of mechanisms that link soil quality and human health through food ingestion needs

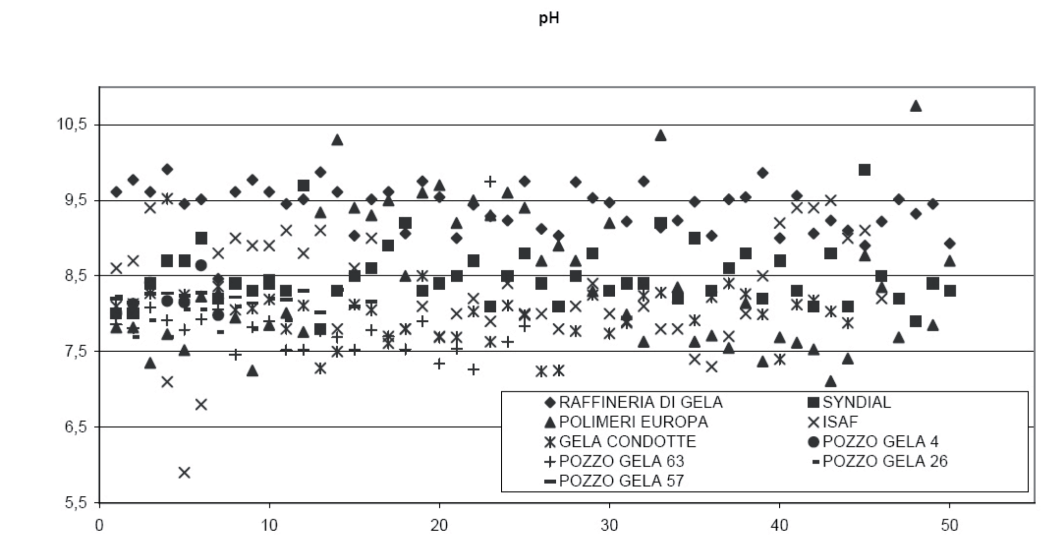

Figure 2. pH variability in soils sampled at the Gela site.

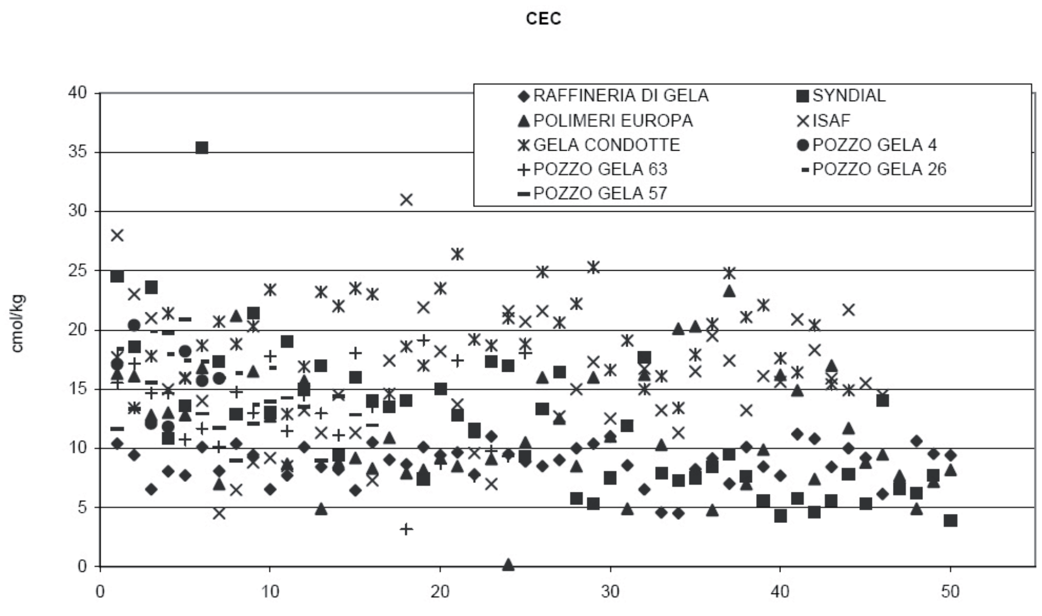

Figure 3. CEC variability in soils sampled at the Gela site.

coordinated and multidisciplinary skills. Such mechanisms are not easy to identify and to address to projects involving a varied skill set.

CNR-ISE's research is aimed at studying the mechanisms of transport and the transformation of contaminants from sources to soil. The dose of contaminants needed to determine biological alterations, which can increase the risk of pathologies is determined on the basis of duration, frequency and intensity of exposure.

The transfer of substances from soil to humans follows complex pathways, in relation to the physical and chemical properties of the soil and to the characteristics of the biological receptor. The transport of contaminants needs to be

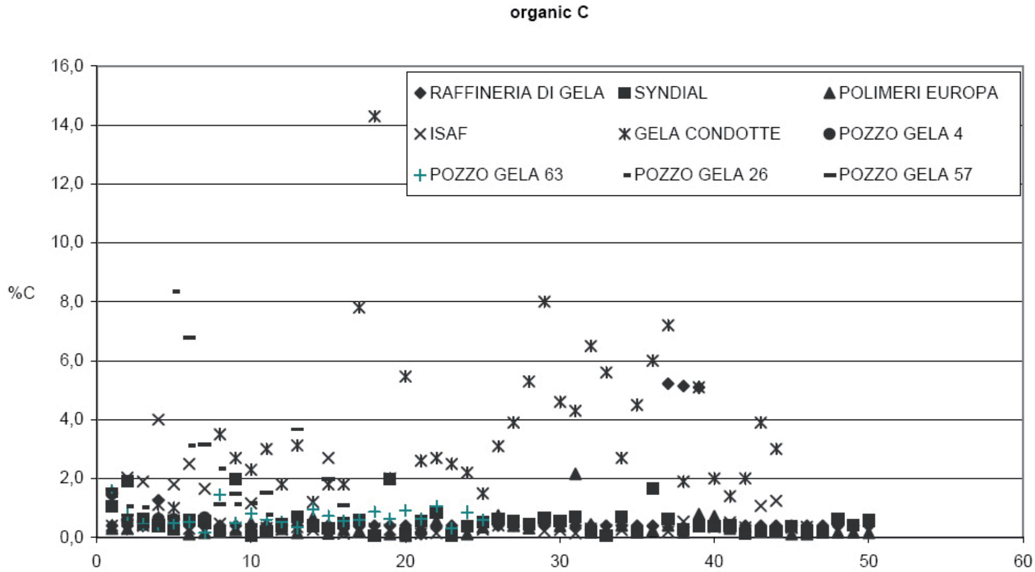

Figure 4. Organic carbon variability in soils sampled at the Gela site.

tackled starting from the highest critical state areas.

The studies at CNR-ISE focus on the effects of chronic exposure to low levels of contaminants, either individual or in mixtures, and on the consequences of exposure to high doses of contaminants naturally present in the environment.

In this particular field CNR-ISE is carrying out studies on the bioaccessibility of contaminants (i.e. heavy metals and selenium) in soil in relation to their bioavailability. Bioavailability is the capacity of a contaminant to interact with the biological world and involves the remediation of contaminated soil using green technologies.

Within this field, the CNR-ISE group has organized national congresses on Soil Quality, Food and Health in cooperation with the Institute of Clinical Physiology (CNR-IFC), University of Bari and the Local Operative Division of Gorizia of CRA-RPS. The congresses were funded by the Italian Ministry of Agriculture and Forestry and the third edition will be held in 2010.

Possible applications of this research

- To identify contaminant pathways in contaminated sites and surrounding areas and the effects of pollutants in soil on dietary uptake.
- To evaluate of the transport of contaminants from sources to target via soil-plant system.

The *Institute of Biophysics* of Genoa (CNR-IBF, Genoa) has a considerable experience in electrophysiology and ion channel biophysics in nervous and endocrine culture cells, investigated by patch-recording and voltage-clamp techniques, and intracellular calcium dynamics, studied by fluorescent probes. In recent years, these skills have been applied to the study of heavy metal accumulation and toxicity in mammalian cells and the modulation of neurotrasmitter-gated ion channels by metal ions in primary neuronal cultures and in recombinant receptors expressed in heterologous systems (frog oocytes and/or mammalian cells). The group has additional expertises in molecular and cellular biology, including PCR and RT-PCR, in vitro transcription and the functional expression of wild type and mutated protein clones in *Xenopus oocytes*, cell culture and mammalian cell transfection. They also study the effect of acute and chronic treatment with heavy metals (Pb, Cd, and others) on cell survival and the maturation of neurons in culture by functional and viability tests and apoptosis/necrosis measurements. Recent work has characterized some Cd and Pb permeation pathways through the neuronal membrane and has identified the location of specific binding sites on the NMDA receptor channel for Pb and Ni.

The CNR-IBF group in Genoa also studies the molecular and cellular mechanisms regulating astrocytic-neuronal interactions in physiological and pathophysiological conditions. This is done using calcium imaging, immunoblotting and electrophysiological techniques. They research the features and roles of P2X7 purinoceptors on primary cultures of neonatal and adult astrocytes, in secondary cultures stably transfected with rat P2X7 or expressing truncated P2X7 receptor, cocultures of neuron/glia and on purified nerve terminals (synaptosomes) and astroglial fraction (gliasomes).

Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants present in the food chain and in human blood and milk. Exposure to PBDEs during pregnancy and lactation leads to signal pathway modifications, calcium homeostasis alteration and apoptotic neuronal death. Such events could play different roles depending on the developmental stage of the central nervous system. Scarce reports are available on specific models, allowing dissection of diverse mechanisms involved and temporal sequences of prenatal or neonatal exposure to PBDEs. Relative contributions of neurons and glia, and their bi-directional communication in the control of glutamate synaptic level and in excitotoxicity triggering and execution, are not clearly defined. Knowledge of the efflux mechanism of the excitatory aminoacids and of how they regulate in the early and late phases of exposure to PBDEs, could lead to the possibility of regulating extracellular excitatory aminoacid levels in different neonatal PBDEs phases. As intracellular Ca^{2+} accumulation seems to be a prerequisite for neuron damage cascade, the dampening of Ca^{2+} influx through ionotropic glutamate or purinergic receptors (e.g. P2X7) could significantly reduce neuronal damage. We therefore plan to investigate the glutamate efflux and cellular Ca^{2+} levels in physiological conditions and during PBDE exposition in vitro models of the neonatal and adult brain.

Parallel studies will be conducted to investigate whether the amino acid release from astroglial cells can be modulated by endogenous signaling molecules through the regulation of swelling-activated Cl- channels. The following experimental models (from the cerebral cortex of neonatal and 60 days-old rats) will be used: i) isolated purified nerve terminals (neuron model) and gliasome (astroglial fraction unpolluted by nerve endings, model for astrocytes ex vivo) from the cerebral cortex of neonatal and adult rats after food exposition to PBDEs insult. ii) in vitro cortical neurons and astrocytes. A functional and pharmacological characterization will be carried on these models out of ionotropic glutamate and purine receptors by studying the "release" of glutamate (or [3H]D-aspartate), and intracellular Ca^{2+} transients with fluorescence methods (Fura-2).

Possible applications of this research

- Identification and validation of cellular models (cultured cells) to establish significant alternatives to in vivo animal tests in toxicology.

- Characterization of metal binding sites on neurotransmitter receptors and other ion channels for designing selective ligands to be used in clinical pharmacology.
- Implementation of biosensors to appraise the bioavailable fraction of toxic metals and to establish the factor of correlated biological risk.

Knowledge of the modes for controlling extracellular glutamate accumulation and cellular Ca^{2+} overload and their relationship with swelling-activated Cl-channels in neonatal and adult brains exposed to PBDE insult would contribute to a rational therapeutic strategy to neuroprotection with a multipharmacological approach. In vitro neonatal models, using controlled experimental conditions and the dissection of pollutant mediated neurotransmitter efflux modes are only at a very early stage of development. Furthermore, little is known about the control of the excitatory neurotransmitter efflux from nerve terminals and the role of neuronal and glial counterparts in the control of glutamatergic transmission and of glutamate level at glutamatergic synapses in the developing and adult brain.

The *Institute of Biophysics* of Pisa (CNR-IBF, Pisa) is involved in the quantification of prospective toxicity in aquatic environments in order to evaluate the relative risk of the introduction of unknown contaminants. Water organisms can be contaminated directly or indirectly. The former occurs by contact or ingestion of the substance dissolved in water, whereas the latter happens when the contaminant is accumulated in the food chain. Chemicals present in sewage of industrial or agricultural origin are liable to contaminate soil, superficial water as well as groundwater aquifers and thereby represent a risk for all water use: drinking water as well as bathing, irrigation and breeding water.

Contaminants may be of a chemical or biological origin. The former mainly consist of substances with slow degradation rates and which are therefore easily accumulated in the soil and aquifers and thus in the first levels of the trophic chain – photosynthetic organisms, plants and algae. Chemical residua, such as pesticides, herbicides and heavy metals, in animal and human tissues undergo a biological magnification process. Their toxicity, often consolidated by a prolonged presence, represents an important health risk. Among the contaminants of a biological origin, those relevant to health issues are algal toxins: these may be released in the aquatic environment and have a toxic effect on humans.

Possible applications of this research

The research group aims to create "early warning" monitoring systems on marine, fluvial and basin waters using microspectroscopy and digital microscopy. In fact, in vivo and in situ microspectroscopic and microfluorimetric measures performed on the photosynthetic compartments of algae present in waters contaminated by either organic compounds and/or heavy metals, reveal the quantitative effects of the pollutants on the chlorophyll:carotenoid ratio and on photosynthetic efficiency. All this information indicates the quality of the water and which of the microalgae analysed may be used either as biosensors or bioremediation, Digital microscopic measures, on the other hand, obtained with optic microscopy techniques and image processing are able to identify and classify algal species (even to identify a single occurrence), to determine water quality and to recognise those species producing toxins dangerous to human health.

The *Institute of Agro-environmental and Forest Biology* of Rome (CNR-IBAF, Rome) has a mushroom germ plasm bank. Their research focuses on the:

- collection and characterization of the wild germ plasm from different countries;
- use of mushrooms for recycling agricultural and agroindustrial waste;
- degradation of lignocellulosic materials for animal feeding;
- biotechnologies for environmental applications of fungi.

To ascertain the quality and the safety of both the substrates and the fruiting bodies, analyses on the presence of xenobiotic substances, in particular the presence of heavy metals have been performed,. Moreover, in the field of the alternative use of mushrooms, technologies have been developed aimed at:

- obtaining polysaccharides through extraction in fruitbodies and in mycelia; fungal polysaccharides, especially chitin and chitosan, are widely applied in pharmacopeia, cosmetics, diets and environmental applications;
- using mycelium biomass for wastewater depuration. Several batch studies have been performed to test the ability of mushrooms to adsorb heavy metals. Fungal biomass loaded PVA was then used in columns for the depuration of water containing heavy metals.
- using mycelium biomass for mycoremediation, in the degradation of phenols, antimicrobial tetracyclines, polycyclic aromatic hydrocarbons and heterocyclic compounds.

Possible applications of this research

Mushrooms can be considered as:

a) living organisms or

b) food.

In case a) mushrooms can be used for mycoremediation in water depuration or in organic molecule degradation. It would be interesting to study the role of polysaccharides present in the cell walls in heavy metal adsorption and the degradation of toxic compounds.

In case b) it is important to consider that mushrooms are able to adsorb heavy metals in the soil in which they live or in the growth substrates used for their cultivation. High concentrations of metals are toxic and inhibit growth and fructification, but with different responses for different metals. It would be interesting to evaluate the dose that permits mycelium expansion and carpophore formation but which may still be dangerous if the mushroom is used as human food.

The *Institute of Methodologies for Environmental Analysis* (CNR-IMAA, Potenza) has considerable expertise in the study of mineralogical and geochemical risks to human health as well as the use of geo-materials for therapeutic treatments (such as pelotherapy and pharmacology).

In recent years CNR-IMAA has applied mineralogical and geochemical information in order to:

- investigate the presence of potential toxic elements in waters and rocks outcropping in some areas at risk and also to study the mobility of some chemical elements due to rock-water interaction processes;
- identify geo-environmental risk factors (temperature, water quality, trace elements, etc.) affecting the biominerals present in the human body (in particular in kidney stones and bones) and their mineralogical and chemical composition.

Possible applications of this research

- The identification of lithologic pollution due to rock-water interaction processes could lead to the production of geochemical maps which could be considered as tools to protect human health.

- The chemical, mineralogical, petrological and textural study, carried out with integrated techniques, can be used to collect useful information on the processes of neo-formation and the transformation of both pathological and non-pathological biominerals present in the human body (stones, osteoporotic bones, teeth etc.).
- The methodological approach involved (epidemiology and geo-environmental features) may enable information to be gathered that would be useful for preventing and treating some diseases.
- The identification of a procedure for characterizing and highlighting the use of mineral sources in paleotherapy and pharmacology.

The research of the *Institute for the Dynamics of Environmental Processes*, (CNR-IDPA, Venice) is focused on studying the environmental processes, especially the mechanisms of transport and transfer of organic and inorganic pollutants, both at local-regional and global levels.

To understand the accumulation and transfer processes along the trophic web, it is essential to study the behaviour of trace elements and persistent organic pollutants (POPs) at a chemical and biological level. In addition their chemistry needs to be considered in concentration terms, as well as variations in the chemical species in which the elements can be present. In order to completely understand the processes and mechanisms that control the involvement of metals and organic compounds at various organisation levels of the ecosystem and the interaction levels between the various trace elements, it is important to closely examine their absorption and transport along the trophic web. The interaction of an element with other parts of a system depends on its chemical form, so it is very important to study the speciation of trace elements and their effects on the interactions between various biotic or abiotic compartments of the environment in depth. As for trace elements, the same can be said for persistent organic pollutants (POPs), which include polychlorobiphenyls (PCBs), polyaromatic hydrocarbons (PAHs), dioxins and hexachloro-cyclohexanes. In addition knowledge of the concentrations of the diverse congeners in the environment plays a key role towards a better understanding of the transport along the trophic web and subsequently the bioconcentration and the biomagnification in biota.

Several studies have been carried out in highly polluted areas and in pristine environments, such as the Venice Lagoon, the Ross Sea in the Southern Ocean, Morocco, Vietnam, Mexico, etc. Different environmental matrices were sampled (seawater, sediments, biota, etc.) and the concentrations of organic micropollutants and of trace elements were assayed; furthermore, the speciation of trace elements were studied. It is known that several organic pollutants, such as PCBs, can bioaccumulate within the trophic web, at a level directly related to environmental levels, and levels within an organism's diet. Therefore for an accurate risk assessment, all the information on congener levels in the biota and the environment has been integrated with the WHO Toxic Equivalent Quantities (TEQs).

The Venice Lagoon represents a particular ecosystem, a transition between two very different environments: the Adriatic Sea and a drainage basin. The variety of inputs (fresh waters, including run-off from agricultural soil and contaminated industrial sites, seawater, industrial and urban wastes and the input of pollutants via aerosol) deeply affect the environment, which is also characterised by a high biodiversity and high productivity determined by the input from the drainage basin. Thus, taking into account the particular features of this environment, the Venice Lagoon is among the areas protected by the European Framework Directive (22nd December 2000, aka Water Framework). According to the Water framework, in-depth studies on the environment are required at a morphological and ecological level. These should study the impact of environmental change taking into account any socio-economic impacts. Of primary importance are methodological studies to develop guidelines to evaluate environmental risks that operate not just at a technical or legal level but also at a scientific level.

Research also focuses on monitoring stress biomarkers in indicator organisms specifically chosen for each environment under study. The environmental monitoring of biomarkers and bioindicators could provide fundamental information. This would contribute towards an improvement of direct measurements, such as chemical measurements by looking at the speciation of potentially toxic elements. Thus for a correct evaluation of environmental risks, analyses need to take a holistic approach. Biomonitoring and chemical measurements need to be integrated, taking into account the diversity and similarities between organisms and between organisms and their environment. This would contribute towards as complete a vision as possible of all the possible transport routes, and all the possible exposure and assimilation modes, as well as bioaccumulation and toxicity dynamics.

The contamination of waters and sediments in coastal areas and harbours is due to a wide range of organic (POPs, such as PCBs, PAHs, etc.) and inorganic pollutants (trace elements, such as mercury (Hg), lead (Pb), chromium (Cr), etc.). In these areas sediments may be a significant sink and/or source of these pollutants. Taking into account the necessity to dredge sediments in order to keep navigation channels open, remediation and environmental recovery are of great consequence in harbour areas. In fact, the management of dredged sediments is crucial for the growth of the port of Venice, due to increasing sea traffic and foreign trade. In view of the dredging of many millions of cubic meters of sediment according to the ‘Piano di Recupero Morfologico’, CNR-IDPA carried out a sediments remediation project (RISED, Azione Biotech III, Regione Veneto), in collaboration with the Venice Port Authority. The main aim of this project was to assess an innovative washing procedure for dredged sediments. The goal was to been environmentally friendly and suitable for the variety of organic and inorganic pollutants, by exploiting the properties of natural organic substances.

Activities, future applications and innovations

Analyses of organic micropollutants are carried out in the CNR-IDPA laboratory, by gas chromatography coupled with high resolution mass spectrometry (GC-HRMS). In addition to the traditional extraction systems, there is a pressurised solvent extraction system (One-PSE, Applied Separations), an automated sample purification system (Power-Prep, FMS) and an automated system for reducing the sample volume (Turbovap, Zymark) in the laboratory of the institute. Two gas chromatographs (HP 6890 Series), with autosamplers, coupled with EI-MS detectors (one is a high resolution detector coupled with a double focus magnetic sector Thermo Finnigan Mat XP 95, the other is a low resolution quadrupole detector HP 5973) enable several classes of organic pollutants at trace and ultra trace levels (PCBs, PAHs, dioxins, emerging hazardous and/or priority substances, etc.) to be determined in different and complex matrices.

Furthermore, an important future aim is to establish a risk assessment for POPs (persistent organic pollutants), which includes information on toxicity and on the accessibility and availability towards biota in a very efficient and pliant way. For a correct evaluation of environmental risks, an assay for stress biomarkers, specifically for the exposure to organic contaminants is being applied to different species of biota.

A class 100 Clean room minimizes any contamination of samples to be analysed for trace elements. Three differently equipped ICP-MS instruments, namely a Thermo Finnigan Element 2, coupled with an autosampler, an Agilent Technologies 7500i coupled with an autosampler, and an Agilent Technologies 7500cx equipped with a collision cell, enable the analysis of elements at trace and ultra trace concentration levels in different matrices (seawater, sediments, air, food, different species of biota). In order to better understand the bioavailability and the bio-accessibility of trace elements, the study of geo-speciation in sediments is carried out using sequential extraction and analysis by ICP-MS. Since the bioavailability of trace elements depends on speciation, it is essential that analytical methods are available to determine or predict the bioavailable fraction of a metal. Thus, the speciation of trace elements such as arsenic (As) and mercury (Hg) is studied using various methods. For a correct evaluation of environmental risks, an assay for the stress biomarkers for the exposure to different species of trace elements is being applied to different species of biota.

The use of new technologies for environmental monitoring is fundamental when planning environmental recovery scenarios in order to appropriately manage particular environments. The results obtained in the RISED project were very promising (both organic and inorganic pollutants appreciably decreased after the treatment), due to the holistic approach used for the various classes of pollutants. Furthermore, dredged sediments are no longer harmfully toxic waste, so they may be a very important resource for recovering the lagoon landscape. This study also underlines the importance of speciation, since according to the most recent frameworks on risk assessment, it is essential to know the bioavailability and bioaccessibility of pollutants in order to plan the most suitable remediation project. Future research includes the possible application of various natural organic substances and the synergy of sediment washing with other remediation techniques, such as bioremediation or phytoremediation.

Throughout the world there is a growing awareness of environmental management, which is associated with careful environmental risk management. The Biotic Ligand Model (BLM) is an important instrument for assessing the risk posed by trace elements. It can be used to determine the bioavailability of a trace element, and the sensitivity of organisms to it as a function of its aquatic chemistry. Thus this a tool for estimating site specific toxicity factors of the elements under question. Future research needs to apply the best “tool-box” according to the Water Framework, so as to establish the environmental risk assessment from a scientific, technical and legislative point of view.

The *Methodological Chemistry Institute* (CNR-IMC, Monterotondo, Rome) has carried out studies concerning the performance of chromatographic and electrophoretic methods for the analysis of different classes of organic pollutants and their degradation products in water, soil and plants and milk. The following classes of compounds have been considered:

- Pesticides (among which are some EDCs such as hexachlorocyclohexane, fenvalerate, linuron, atrazine)
- Phenols
- PAH
- VOCs (Benzene, Toluene, Xylene , aliphatic ketones and alcohol in goat milk)

In recent bioremediation experiments at IMC, the distribution of hexachlorocyclohexane isomers in soil, plants and rhyzosphere has been studied, together with the degradation of such compounds by bacterial strains inoculated in poplar plants in a greenhouse experiment.

The degradation of PAHs and phenols by ligninolytic fungi and the interaction of PAH metabolites with humic acids have also been studied.

Possible applications of this research

Interaction of organic pollutants and their metabolites with humic matter and dissolved organic matter in soil and water.

Identification of biodegradation compounds of organic pollutants in soil by bacteria and fungi and uptake by plants.

Transfer of VOC from the environment to mammalian and milk contamination

4.2 External collaborations

The *Department of Biology and Chemistry of Agro-Forestry and Environment* at the *University of Bari* (DIBCA, Bari) is mainly involved in various studies related to soil chemistry and biochemistry, such as:

- the monitoring, conservation and improvement of soil fertility in order to maintain soil quality and increase crop productivity;
- the role of soil in complex biogeochemical cycles also disturbed by anthropogenic impacts (spillings, amendments, disposal, reclamations, etc.);
- the processes and techniques of soil and sediment decontaminations from heavy metals and various xenobiotics;
- the physical, chemical and biological indicators for soil health and quality in response to internal and external anthropogenic activities;
- the environmental risks deriving from the agricultural use of genetically-modified organisms (GMO).

These objectives are achieved through a complex theoretical and experimental approach involving simultaneous chemical, physical and biological phenomena and processes occurring in the soil-plant-xenobiotics system. With given environmental conditions, this consequently leads to a correct and rational use of natural soil resources balancing soil productivity and soil protection.

Possible applications of this research

Monitoring heavy metal and organic pollutants through the food chain, especially in the soil-plant system: relationships between pollutants and soil organic components and related fractions; degradation and retention phenomena of conventional and organic pesticides in various soil systems.

Evaluation of the physico-chemical transformation of organic components in relation to organic waste amendments._

5. Future perspectives and developments

Millions of chemicals are released into the environment, and end up in the soil; the impact of most of them on human health is still not fully known. There are also naturally occurring amounts of potentially toxic substances in the soil whose fate in the terrestrial environment is still poorly known. The behavior of contaminants in soil is related to both the contaminants and the characteristics of the soil. The soil properties regulate the distribution of a substance among the soil phases (solid, liquid, gaseous) and thus determine the retention, the release and the migration of each contaminant (26, 62, 102, 107).

Pollution is also regulated by a time factor, which influences the availability from the source emission to the final target. Human exposure to soil pollution is therefore time-dependent both directly and through secondary transfers such as the food chain.

Unlike our knowledge of the exposure of soil to water and air pollutants, our understanding of the effects of soil contamination is still in its early stages, due to the numerous reactions that take place in the soil (sorption, release, degradation, ageing). These reactions modify the bioavailability of contaminants, which is dependent on the specific characteristics of each particular soil. All these reactions are also influenced by natural weathering processes, which contribute to the transport and the erosive migration of contaminants.

As a result, and despite several legislations regarding soil, models of pollutant behaviors are very poorly defined, especially in terms of the terrestrial food chain. The transfer of contaminants from soil to the food chain requires a detailed knowledge of the complex reactions, that influence their bioaccessibility and these reactions are completely different from those defining the source of exposure.

A study of the fate of contaminants in the soil can thus provide a reasonable estimate of exposure. This can then be used as a basis on which to evaluate adverse effects on human health (4, 56, 79, 94). There is still a great uncertainty regarding this issue and the need to tackle the following issues has been recognized at an international level:

- the dietary uptake from vegetables grown in polluted soils;
- accidental soil ingestion;
- bioaccessibility and bioavailability.

Most attention is now focused on the last issue, since the bioavailability of contaminants in soil (65, 71) is quite different from that deriving from the toxicological experiments on which the risk assessment is founded. Therefore only a deeper understanding of the characteristics of the soils that regulate the chemical and biological reactions of contaminants will contribute to decreasing the uncertainty in risk assessments (36, 41, 79, 112), and the consequences of soil pollution on human health.

One of the main objectives of PIAS-CNR project "Environment and Health" was to highlight the close links between environmental matrices and human health. Within this framework, Working Group 1 (WG 1) focused attention on the fate of contaminants in the environment, particularly on the soil ecosystem. The fate of contaminants in the soil depends not only on the original chemical form of the contaminants, but also on the specific characteristics of the soil. The ability to accurately determine the effects of contaminants on individual species, populations, communities and ecosystems is hampered by an uncertainty in the quantification of receptor exposure pathways. Laboratory and field studies have shown that hazards for human health did not derive from the total concentration of a contaminant in the soil, but from the fraction that is biologically available for that population at that specific time and with certain soil conditions. It is now common knowledge that total concentrations are not useful to explain the effects of contaminants. These effects may differ from soil to soil depending on soil characteristics and environmental conditions. However one of the main shortcomings of the present procedures to evaluate the risks for human health is the inadequacy, or total absence, of incorporating the bioavailability of contaminants.

Half of a century ago, total diet studies were initiated in response to concerns regarding the loss of food quality. Nowadays the list of foods analyzed is continuously updated and through the database of U.S. Food and Drug Administration (FDA) it is possible to know the content of pesticides, heavy metals, dioxins and other contaminants in many foods and beverages. These studies represent a suitable tool for monitoring dietary intake both in industrial and in developing countries. Many studies have also been carried out in Italy and it appears that most of the elements in the Italian total diet derived from plant foods (88).

The WG 1 proposal aims to go beyond total diet studies and to understand mechanisms and processes by which contaminants enter the food chain and influence to various extents nutrition and the health of humans.

The general objective of this proposal concerns the establishment of a knowledge network among all the PIAS Working Groups. This will at the same time enable each WG to promote and conduct research strategies in medical, especially epidemiological circles. However, it is necessary to devote the same attention to soil pollution as has been previously given to air and water pollutions. In this respect, bioavailability processes assume an essential role to efficiently describe the occurrence of contaminants in food, the effects of soil quality on the quality of food products, and the implications for human health.

The working path of the WG 1 Proposal project starts with the study of contaminant bioavailability in soil. Bioavailability can be defined as the degree to which chemicals present in the soil matrix may be absorbed or metabolised by human or ecological receptors or are available for interaction with biological systems.

In addition, bioavailability depends on time. Due to ageing, contaminants binding to the soil may become stronger, consequently reducing the effects on the environment. On the other hand, due to natural or anthropogenic changes in soil factors (e.g. pH) contaminants may become more available. The task of soil chemistry is to define the available fractions, the potentially available fractions, and the non-available fractions of contaminants in different environmental conditions.

The proposal is to study contaminant bioavailability in three geographical areas characterized by soils of different origins where it is possible to find either areas with a high degree of pollution due to contamination sources (known as "Site of National Interest: SIN"), areas characterized by natural high levels of metals (Cr, As, Hg), and areas characterized by the absence of point source pollution.

The general aim of this proposal is to identify the transfer of contaminants from soil to the food chain and to evaluate the possibility that in highly polluted areas, soil might not be able to exert its essential role as a filter for ground waters.

The outcome of this part of the project is also to produce a model or to examine the possible use of an existing model (such as the Dutch CSOIL model) to evaluate the routes through which individuals are exposed to a given pollutant. The main routes to consider are: ingestion of soil by children, inhalation of soil dust, and the consumption of vegetables. This evaluation must be carried out by inserting bioavailability concepts in the models to obtain reliable data, which would also be useful in deriving the concentration of a contaminant in a specific soil in relation to human health.

All the research centers mentioned in Chapter 4 will provide their own contribution throughout the whole pathway of the project (Figure 5) under the coordination of CNR-ISE (Pisa).

The first step is to detect the pollutant entity (structure, agent) in soil and water. CNR-ISE (Pisa), will provide an identification of contaminant pathways and an evaluation of the transport of contaminants from sources to target via soil-plant systems. DIBCA (Bari) will provide the monitoring of heavy metal and organic pollutants through the food chain, as well as the study of the relationships between pollutants and organic soil components and related fractions. Through the identification of geo-environmental pollution due to rock-water interaction processes, CNR-IMAA (Potenza) will create geochemical maps for use as tools to protect human health.

The second step is to understand the mechanisms which influence the transport of chemicals in the soil-plant system. Using microspectroscopy and digital microscopy, CNR-IBF (Pisa) will examine the transport, diffusion and accumulation processes of pollutants from the accumulating matrix (soil and water) to plants and animals. CNR-IDPA (Venice) will focus on environmental processes, especially the transport and transfer of organic (polychlorobyphenils, polyaromatic hydrocarbons, dioxins and hexachloro-cyclohexanes) and inorganic (trace elements, such as mercury, lead, chromium, etc.) pollutants. CNR-IDPA (Venice) is involved in the analysis of organic micropollutants, using gas-chromatography coupled with high-resolution mass spectrometry and elements at trace and ultra trace concentration levels in different matrices with three differently equipped ICP-MS instruments. CNR-IMC (Rome) will focus on the analysis of different classes of organic pollutants and on the identification of compounds subjected to biodegradation in soil by bacteria and fungi, and to uptake by plants. CNR-IBAF (Rome) investigates the ability of mushrooms to absorb heavy metals in soil. Mushrooms can be used for mycoremediation in water depuration or in organic molecule degradation.

The third step is the study of pollutant bioavailability in order to plan the most suitable remediation strategy. ISE-Pisa will study the bioaccessibility of contaminants in soil in relation to their bioavailability. CNR-IDPA (Venice) will use analytical methods to determine or predict the bioavailable fraction of a metal.

The fourth step is an investigation on the transfer of pollutants from soil to humans through the food chain. CNR-IBF (Genoa) will focus on the characterization of metal binding sites in neurotransmitter receptors and other ion channels for the design of selective ligands to be used in clinical pharmacology. CNR-IBF (Genoa) will also study the implementation of biosensors in order to appraise the bioavailable fraction of toxic elements and to establish the correlated biological risk factors. A comprehension of the control mechanisms of neurotransmitter receptors and ion channels in the neonatal and adult brain exposed to a specific class of organic pollutants (polybrominated diphenyl

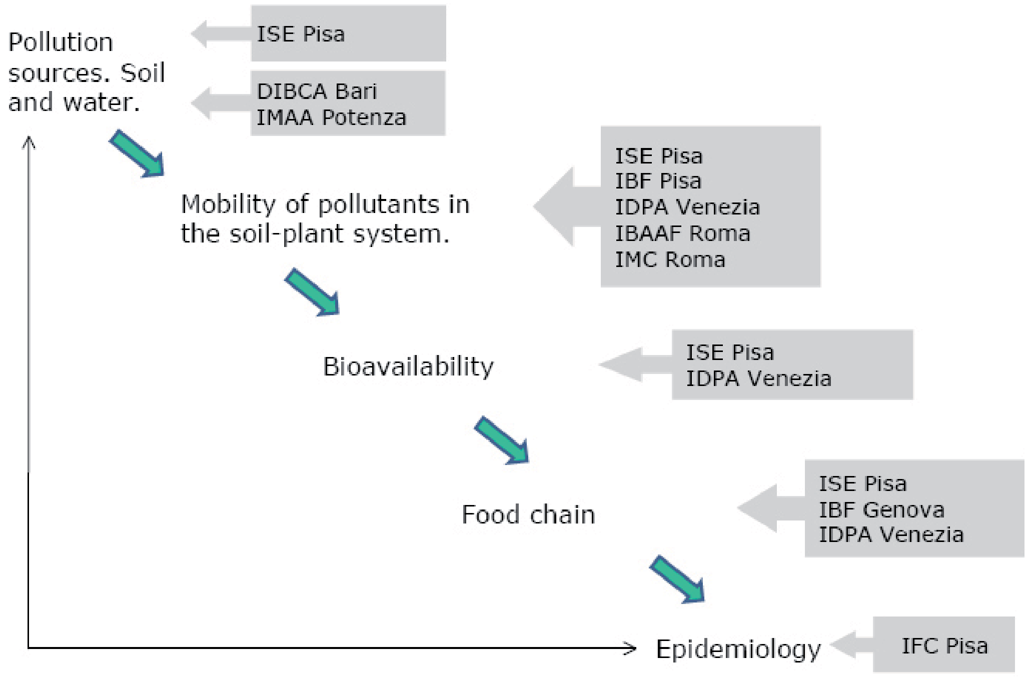

Figure 5 – Project proposal

ethers) would allow a rational therapeutic strategy in a multipharmacological approach to neuroprotection. CNR-IDPA (Venice) will focus on the absorption and transport of metals and organic compounds along the trophic web. Knowledge of the concentration of the different congeners in the environment plays a key role in understanding bioconcentration and biomagnification in biota. The chemical, mineralogical, petrological and textural study, carried out with CNR-IMAA (Potenza) integrated techniques, will enable useful information to be gathered on the processes of neo-formation and transformation of both pathological and non-pathological biominerals present in the human body (stones, osteoporotic bones, teeth etc.). The methodological approach involved (using epidemiological and geo-environmental features) may provide useful information for preventing and treating some diseases.

All the previous steps will be completed by an integration of epidemiological studies carried out by CNR-IFC (Pisa).

With the contribution of WG1 participants: F. Bianchi (IFC), W. R. L. Cairns (IDPA), P. Cescon (IDPA), F. Corami (IDPA), L. Cori (IFC), E. Galli (IBAF), P. Gualtieri (IBF), C. Marchetti (IBF), T. Miano (Univ. Bari DIBCA), M. Nobile (IBF), R. Piazza (IDPA), C. M. Polcaro (IMC), V. Summa (IMAA)

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