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Risk assessment and mitigation measures for certain amendments used in the bioremediation of sewaged soils

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Abstract: The limited water resources in Egypt led to the use of sewage effluent in farming. After two years R&D activities targeting bioremediation of sewaged soil ecosystems, a major chain protocol comprised of some remedial chemical and biological additives' together with some single protocols were reached. The precautionary and sustainable approaches in sewage farming unquestionably necessitate ensuring safety during and after the application of these reached bioremediation protocols. To achieve an accepted safely level, risk assessment was initiated as an effectual tool recognizing and weighing up the potential adverse impacts of the reached bioremediation protocols and to advice whether or not they are acceptable or manageable. However, it should be clarified that lack of scientific knowledge or consensus does not automatically be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk. Together with that it is worthy to mention that sustainability occurs only when the productive potential of a sewaged soil ecosystem continues for a long time. Within this insight, the key intent of this was to attain sustainable farming after bioremediation of the sewaged soil ecosystem through combating and mitigating the adverse impacts of the reached protocols on the basis of the results of risk assessments. This paper, however, provides information that is broadly relevant to the risk assessment of the reached protocols concerning their intended uses within the scope and objective of sustainable sewage farming.
Key words: Bioremediation, sewaged soils, mitigation measures, bentonite, rock phosphate, phosphate dissolving bacteria, mycorrhizae, phytoremediation, Thiobacillus sp., calcium hypochlorite.

1. Introduction

Risk assessment is the determination of quantitative and/or qualitative value of risk related to a concrete situation and/or a recognized threat. In the context of the environment, risk assessment is the process of quantifying the probability of any adverse effects to human population and biodiversity from certain activities [1]. The application of sewage effluent in farming as well as the use of certain remediative amendments in bioremediation of sewaged soils might bear a potential dispersal of a wide range of contaminants to agricultural soils. These contaminants might be further transported to different environmental compartments such as air, surface water, ground water and nearby streams. Furthermore the contaminants in soil might invade into food chain through absorption by plants [2].

In the estimation of risks, three or more steps are involved that require the inputs of different disciplines, the first is the hazard identification that aims to determine the qualitative nature of the potential adverse consequences of a contaminant (chemical, physical, biological, etc.) and the strength of the evidence it could have such effect, the second is the dose-response analysis that determine the relationship between dose and probability and/or incidence of effect (dose-response assessment), the complexity of this step in many contexts derives mainly from the need to extrapolate results from experimental animals (e.g. mouse, rat) to humans, and/or from high to lower doses, the third is the exposure quantification that aims to determine the amount of a contaminant (dose) that individuals and populations will receive. These all are done by examining the results of the discipline of exposure assessment. As far as different location, lifestyles and other factors are likely to influence the received amount of contaminant, a range or distribution of possible values must be generated in this step. Particular care might be taken to determine the exposure of the susceptible contaminants [2, 3, 4,5].

2. Methods

Risk assessment is a cautious scientific process evaluating what could cause injury to health and/or ecosystem depending on how much of a remedial amendment will be added and how long exposure a person or receptor has with them and their inherent adverse impacts. Ecological and health risk assessment was estimated in the current work according to the guidelines given [6,7,8] as described [9,10,11]. Worldwide, the habitually used ecological risk assessment framework is the EPA, which is dissimilar from the WHO one. However, there are copious returns to their integration, as they both afford more reliable competence from sharing data and potential for assessing the impacts of ecological injuries on human health and wellbeing [11]. Risk assessors evaluated the full range of discernible human health and ecological dimensions of risks in successive integrated steps and iterative manner, as appropriate.

2.1. Risk assessment paradigm

Four major steps in the risk assessment paradigm were studied in the current work as follows:

2.1.1. Context of the problem formulation was a starting iterative step that was laid down through scoping the intent of the risk assessment, identifying and characterizing the proposed remedial amendments associated with the reached protocols and setting endpoints. The intent of the risk assessment included problem narration and identification of relevant goals. Identification of the proposed remedial amendments was not only limited to their characterization but extended to describe their potential functions in the receiving sewaged soil ecosystems whether being direct or indirect, immediate or delayed, combinatorial or cumulative and/or predicted or unpredicted. The end points were set within the frame of the results gained from the project experiments as well as related data available in the literature.

2.1.2. Characterization of adverse impacts was not only limited to identifying hazards of the adverse impacts of the proposed remedial amendments, but extended to link them with the indented goals and endpoints. The levels of adverse impacts and consequences were expressed in terms as major, intermediate, minor, marginal, highly likely, likely, unlikely or highly unlikely.

2.1.3. Risk characterization was the phase of risk assessment that culminated all work done. Through gained results, assessors described and estimated risks posed to ecological entities and human health within their uncertainties context in a frame of transparency, clarity, consistency and reasonableness. Risk characterization considered all expected risks associated with the use of the proposed remedial amendments that might give rise to adverse impacts when interacted with the receiving sewaged soil ecosystems whether being direct or indirect, immediate or delayed, combinatorial or cumulative, predicted or unpredicted, high, medium or low, negligible or indeterminate, acute or chronic, severe or mild, fast or low acting and/or directed to one or many species.

2.1.4. Mitigation measures were included whether or not risks are accepted, evaluated whether the set objectives and criteria were met, and set mitigation measures strategies to combat the adverse impacts of the proposed remedial amendments through avoidance, additional measures and/or contingency plan**.**

The ecological risk assessment for the sewaged soil ecosystem considered risks from existing amendments, remedial actions themselves, residual impacts and subsequent land uses. The ecological and health risk assessment were set through several routes of exposure over varied time frames.

During the risk assessment, assessors focused on two options, the first if risks are unsafe, risk mitigation measures on any or all uses of remedial amendments were set up to ensure conformance with exposure standards for any acceptable risk, and the second if risks are already within the acceptable risk standards, little or no action would be taken.

In cases when an element of risk assessment sounded vital but no valid data were available, the assessors highlighted this deficiency and used judgment and/or assumed values to approximate the missing data. Such judgments and approximations were noted and clearly explained in the risk characterization.

3. Results

3.1. Reached Protocols

After two years R&D activities aiming at bioremediation of sewaged soil ecosystems, the research team of the project reached one major sequence protocol and a single chemical one. The reached major protocol included two main phases, the first phase lasted for two months through treating the contaminated sewaged soil ecosystem with a conglomerate of chemical and microbial remedial additives' persuasive enough to remediate PTEs and POPs and the second phase was phytoremediation of the soil ecosystem with canola plant during winter season, Corn and black nightshade plants during summer season. The single remediation protocol was disinfecting infectious pathogens in the sewaged soil ecosystem by calcium hypochlorite.

Table 1. Potential functions of the used remedial amendments.

Remedial amendment	Type of action	Potential function
Bentonite	indirect, mediate: cumulative and predicted	Adsorb PTEs
Rock phosphate	Indirect, immediate, cumulative and predicted	Increasing available phosphate in soil and hence PTEs
Arbiscular mycorrhiza includes (AM)	Indirect delayed, cornbinatorial and predicted	Maximizing the uptake of PTEs and PCPs by the hyperaccurnulating plants
Bacillus megaterium var *phosphaticum*	Indirect, delayed, cumulative. and predicted	Dissolve tri-calcium phosphate changing it to mono and di-calcium phosphate available to plant uptake
Thiobacillus thiooxidans	Indirect, delayed, cumulative and predicted	Oxidizing elemental sulphlier forming sulphuric acid
Thiobacillus ferrooxidan	Indirect, delayed, cumulative and predicted	Oxidizing elemental sulpher forming sulphuric acid
Canola hyperaccumulator	Direct,delayed,	Removing PTEs and PCPs from soil
Corn hyperaccumulator	Direct,delayed, cumulative and predicted of unpredicted	Removing PTEs and PCPs from soil ecosystem
Black nightshade hyperaccumulator	Direct,delayed, cumulative and predicted or unpredicted	Removing PTEs and POPs from soil ecosystem
Calcium hypochlorite	Direct, immediate arid predicted	Decontamination of infectious pathogens in soil ecosystem

The remedial amendments proposed in the reached protocols (Table 1) comprised of chemical and biological remedial additives, included hyperaccumulator plants and microorganisms. The risk assessment outputs for the proposed remedial amendments showed some potential adverse impacts, therefore, the assessors were faced with the task of determining what risk-mitigation measures might be needed.

3.2. Ecological and health risk assessment

Health risk assessment is a mean of quantifying the probabilities of risky impacts to individuals or populations from certain human activities. It had been regarded as an alternative to ecological risk assessment on the hypothesis that if you protect humans, non-human receptors will be protected as well. For an array of basis, some non-human species are likely to be more exposed to ecological adverse impacts than humans, or might be more perceptive to certain remedial amendments. Health risk assessment differs from ecological risk assessment in quite a few significant ways. For ecosystems, ecological risk assessments must regard impacts beyond just individual organisms or a single species. For public health all humans are treated equally, while with ecosystems, some sites and types are more precious and vulnerable than others.

Sometimes, precautionary principle is wished-for as an option to risk assessment, but like most ecological principles, it has many definitions and applications. The most extreme precautionary principles need ultimate verification of safety before allowing any remediation technologies or actions [12]. Actions related to precautionary principles should be synchronized or existing adverse impacts mitigated if they raise threats of harm to health or ecosystem [13].

3.3. Context of the problem formulation

3.3.1. Scoping the intent of risk assessment:

The current ecological and health risk assessment aims to identify and evaluate the potential adverse impacts of the proposed remedial amendments and set strategies aiming to mitigate these impacts taking into account the uncertainties surrounding the validity of input data.

The current project tackled the problem of bioremediation of sewaged soil to combat the adverse ecological and health impacts resulting from soil ecosystem contamination with PTEs, POPs and infectious pathogens.

The main relevant goals of the risk assessment were reaching acceptable working mitigation measures to combat the expected adverse ecological and health impacts of the proposed remedial amendments both solely and cumulatively.

3.3.2. Identifying and characterizing the proposed remedial amendments

Identification of the proposed remedial amendments was not only limited to their characterization but also extended to describe their potential functions in the receiving sewaged soil ecosystems (Table 2) whether being direct or indirect, immediate or delayed, combinatorial or cumulative and/or predicted or unpredicted.

Table 2. Rate of application of the remedial amendments.

Remedial amendment	Rate of application
Bentonite	1% [3500 kg/fed (4200 m^2)]
Rock phosphate	1% [3500 kg/fed (4200 m^2)]
Arbiscular mycorrhiza includes (AM)	AM fungi spores were extracted from soil by wet sieving[14]. Seeds were inoculated with 20ml of a suspension of mycorrhizal conidia (10^6 CFU) prior sowing.
Bacillus megaterium var. phosphaticum (PDB)	PDB were grown in Bioflo & Celligen fermentor/bioreactor [15] in medium as modified [16] to reach 10^6 CFU before being impregnated on peat moss at the rate of 20 ml microbial suspension per 100 gm mordant oven dried soil and was used as seed coater prior sowing.
Thiobacillus thiooxidans	*Thiobacillus thiooxidans* was grown in Bioflo &Celligen fermentor/bioreactor in modified Waksman medium [17],18. From the culture broth, 10 ml aliquot was sampled every 7 days during incubation to determine its pH until reaching 2 to reach (10^6 CFU) before being impregnated on peat moss at the rate of 20 ml microbial suspension per 100 gm mordant oven dried soil and was used as seed coater prior sowing.
Thiobacillus ferrooxidans	*Thiobacillus ferrooxidans* was grown in Bioflo & Celligen fermentor/bioreactor in DSMZ medium 882 [19]. From the culture broth, 10 ml aliquot was sampled every 7 days during incubation to determine its pH until reaching 2 to reach (10^6 CFU) before being impregnated on peat moss at the rate of 20 ml microbial suspension per 100 gm mordant oven dried soil and was used as seed coater prior sowing.
Canola hyperaccumulator	2.1 Kg seed/fed (4200 m^2)
Corn hyperaccumulator	20 Kg seed/fed (4200 m^2)
Black nightshade hyperaccumulator	15 Kg seed/fed (4200 m^2)
Calcium hypochlorite	500 ppm

3.3.2.1. Bentonite

Bentonite is a rock composed mainly of the clay mineral montmorillonite and might contain in certain sites feldspar, cristobalite, and crystalline quartz. Bentonite is not poisonous and had never been absorbed by a human or animal body. It is characterized by its ability to absorb large quantities of water besides having high cation exchange capacity. These properties are derived from its crystal structure which is composed of an octahedral alumina sheet between two tetrahedral silica sheets. It is identified and quantified by X-ray diffraction, electron microscopy, energy-dispersive X-ray analysis, differential thermal analysis, electron diffraction, and infrared spectroscopy [20]. The difference between bentonite and other clay minerals lies in their crystal lattice structure. The sheet of atoms in bentonite is much thinner and more easily separable in water rendering it able to occupy more surface area than other clay minerals. This property is known as dispersibility, which is unique to bentonite as an extremely potent tool capable of absorbing most PTEs and hence lowering their activities in sewaged soil ecosystems. Bentonite clay is also an effective binding detoxifier acting like a magnet that attracts the positive charges of POPs by its negative charge. Bentonite could also absorb numerous toxic substances, impurities, poisons, pesticides, pathogens, parasites, etc. It does this without posing any nasty side effects.

3.3.2.2. Rock phosphate

Rock phosphate is a natural resource formed of calcium phosphate, mostly called *phosphorite*. Phosphate compound in rock phosphate exists in the form of mineral apatite with widely different chemical and crystallographic characteristics and physical properties. Factors that influence the agronomic effectiveness of rock phosphate are reactivity of the rock phosphate, soil properties, climate conditions, crop species and management practices.

High carbonate substitution for phosphate in the apatite crystal structure, low content of calcium carbonate as accessory mineral and fine particle size (less than 0.15 mm) enhance the reactivity of rock phosphate and their agronomic effectiveness. High phosphate retention capacity of soils might facilitate the dissolution of rock phosphate but the availability of dissolved phosphates will depend on the concentration of phosphate maintained in solution. Application time could be nearer to planting in very acid soils (pH < 5.5) and 4-8 weeks before planting in less acid soils. It is possible to utilize several means to improve the agronomic effectiveness of rock phosphate under a particular set of condition. Selecting the appropriate process requires a good understanding of the factors hindering the agronomic effectiveness. The biological means, e.g. phospho-composting, inoculation with AM, use of phosphate solubilizing micro-organisms as well as use of P-efficient plant genotypes are based on the production of organic acids to enhance rock phosphate dissolution and phosphate availability to plants, and they hold good promise. The use of chemical means to produce partially acidulated rock phosphate is the most effective way of increasing the agronomic effectiveness of rock phosphate. There is a poor relationship between surface area and the solubility of rock phosphate [21]. Application of rock phosphate should consider their quality in terms of total phosphorus pentoxide content, particle size distribution, and solubility. The appropriate and sound application of rock phosphate might significantly contribute towards sustainable agricultural intensification.

3.3.2.3. Arbiscular mycorrhiza (AM)

Arbiscular mycorrhiza is a symbiotic mutualistic fungus belonging to the genus *Amanita* occasionally weakly pathogenic associative with other fungus and roots of vascular plants 16. AM, formerly known as vesicular-arbuscular mycorrhizas or VAM, are mycorrhizas whose hyphae enter into plant cells, producing structures that are either balloon-like (vesicles) or dichotomously-branching invaginations (arbuscules). The fungal hyphae do not in fact penetrate the protoplast (interior of plant cell), but invade the cell membrane. The structure of the arbuscules greatly increases the contact surface area between the hypha and the cell cytoplasm to facilitate the transfer of nutrients between them. AM is commonly divided into ectomycorrhiza and endomycorrhiza. The two types are differentiated by the fact that the hyphae of ectomycorrhizal fungi does not penetrate individual cells within the root, while the hyphae of endomycorrhizal fungi penetrate the cell wall and memberane. In an AM association, the fungus colonizes the host plant's roots, either intracellularly or extracellularly. They are an important component of soil biology and chemistry. While only a small proportion of all species had been examined, 95% of those plant families are predominantly mycorrhizal. The plant gains benefits of the mycelium's higher absorptive capacity for water and mineral nutrients due to the comparatively large surface area of mycelium: root ratio, thus improving the plant's mineral absorption capabilities [22, 23].

Plant roots alone might be incapable of taking up phosphate and PTEs ions that are de-mineralized in soils with a basic pH. AM could access phosphorus and PTEs in soils and make them available to plants they colonize [24]. The mechanisms of increased absorption are both physical and chemical. AM mycelia are much smaller in diameter than the smallest root, and thus could explore a greater volume of soil, providing a larger surface area for absorption. Also, the cell membrane chemistry of AM is different from that of plants including organic acid excretion which aids in ion displacement. Mycorrhizal plants are often more resistant to diseases, such as those caused by microbial soil-borne pathogens, and are also more resistant to the effects of drought. AM had been found to had a protective role for plants rooted in contaminated sewaged soils with high PTEs concentrations. Plants inoculated with AM and grown in contaminated soils displayed high tolerance to the prevailing contaminants, survivorship and growth. Several studies discovered the existence AM strains with varying tolerance of zinc which might probably be due to binding of the PTEs to the mycelium of the fungus, without affecting the exchange of beneficial substances [25].

3.3.2.4. Bacillus megaterium var. phosphaticum

Bacillus megaterium var. phosphaticum is a bacterium able to dissolve fixed phosphate into plant utilizable form. It produces penicillin amidase, organic acids (e.g., citric, succinic, lactic ac, etc.), hormones (e.g. indole acetic acid, gibberellins etc.) and enzymes (e.g., phytase, nuclease, lecithinase, amino acid dehydrogenises etc.) that help in solubilisation of insoluble phosphates into plant utilizable form, decomposing POPs and antagonizing infectious pathogens.The bacteriumis one of the largest eubacteria found in soil, it is rod-shaped, Gram-positive, endospore forming, arranged into the streptobacillus form, aerotolerant species are repeatedly used as a soil inoculants in agriculture and horticulture. Groups of these bacteria are often found in chains where cells are joined together by polysaccharides on the cell walls. *Bacillus megaterium* var *phosphaticum* is able to survive in some extreme conditions such as desert environments due to the spores it forms. The total content of phosphorus in soil could not change very easily and its conversion from not easily accessible forms into readily accessible ones is tightly connected to the microbial activity, chemical reactions, moisture, temperature and soil type. Microorganisms have an important role in phosphorus conversion in soil. Phosphorus could be found in the chemical structure of microorganisms as phosphates. Based on their phosphorus nutrition, microorganisms had been classified to bacteria assimilating to the same extent of mineral and organic phosphorus, bacteria assimilating particularly mineral phosphorus, bacteria assimilating particularly organic phosphorus, bacteria solubilising phosphorus in the form of glycerophosphate, bacteria solubilising tricalcium phosphorus. This bacteria grouping is characteristic for every type of soil. Phosphorus is used differently by the various microorganisms due to their participation in the processes of solubilisation, mineralization and taxation of phosphates in the soil. There is evidence that the chemical nature of most of organic phosphorus in soil is of microbial origin. *Bacillus megaterium var. phosphaticum*also solubilise phosphates by decreasing the pH in the surrounding soil ecosystem or acting on calcium, iron, aluminium, and magnesium salts. It is repeatedly used in producing a bio-agent used to enhance mineral phosphorus solubilisation. If phosphorus is present in the complex structures of soil and at the same time readily decomposable carbon sources, such as manure, are incorporated in soil, phosphorus solubilisation could be increased due to biological activity stimulation [26].

3.3.2.5. Thiobacillus thiooxidantsis

*Thiobacillus thiooxidants*is a strictly aerobic, obligate autotrophic bacterium free from chlorophyll including few species that could grow on organic compounds. They are Gram-negative rod shaped motile with polar flagella, non-spore forming, and mesophilic with a cell size ranging between 0.1- to 3-mm, able to oxidize sulfide, elemental sulfur, thiosulfate, and polythionite. The five most described members of the *Thiobacillus* species are *Thiobacillusthioparus, Thiobacillus denitrificoulds, Thiobacillus thiooxidans, Thiobacillus intermedius, and Thiobacillus ferrooxidans.* Their best growth was noticed at 25-35°C and under neutral pH; however, some species are able to live in highly acidic environments. The genus *Thiobacillus* is responsible for elemental sulfur oxidation yielding 236 kcal of energy. *Thiobacillus thiooxidants* was found at intensities reaching 10^8 cells/gm soil or more wherever reduced sulfur compounds are available [27,28]. Their presence might often be evident to naked eye through the appearance of copious white deposits of sulfur, streamers, veils, rosettes, or films. There is a decrease in soil pH reaching 0.5-2.5 unit after providing the soil with oxidizable substrate like sulfur or pyrite along with inoculation of various strains of *Thiobacillus* cultures [29]. As soil pH decreased, sulfate levels were raised in soil along with concomitant increase in solubilization of PTEs. The formation of H_2SO_4 in soil following additions of elemental sulfur and *Thiobacillus thiooxidans* augments PTEs mobilization [30].

A wide variety of soil microorganisms are involved in sulpher oxidation, in which *Thiobacillus* bacteria are the most important and common sulpher-oxidizing in agricultural soils. Studies on the isolation and selection of microorganisms with ability to promote sulpher oxidation in soil and in turn higher solubilization of phosphoric rocks had been repeatedly carried out. The beneficial effects of application of apatite along with sulpher and its oxidizing bacteria (*Thiobacillus*) to enhance nutrients and PTEs availability and in turn their uptake by plants had been repeatedly noticed.

3.3.2.6. Thiobacillus ferrooxidan

Thiobacillus ferrooxidans is an obligate rod-shaped, mesophilic, acidophilic, autotrophic, chemolitho trophic, strictly aerobic, colorless, rod-shaped, Gram-negative, non-spore forming bacterium, motile with a single flagellum & pili or non-motile occurring in single or occasionally in pairs or chains. It is adapted to wide variations of soil temperature and pH range and could be readily isolated and enriched in many soil ecosystems. It is a potent leaching bacterium in PTEs dissolution in soil ecosystems. *Thiobacillus ferrooxidans* derives its biosynthetic requirements by autotrophy using carbon from atmospheric carbon dioxide. Nitrogen fixation also is an important ecological function carried out by this bacterium in acidophilic habitats. Metabolic energy is derived aerobically by the oxidation of reduced inorganic sulfur compounds or ferrous ions. Anaerobic growth using elemental hydrogen or reduced inorganic sulfur compounds as electron donors and ferric ions as electron acceptors had also been noticed. Although *Thiobacillus ferrooxidans* classified as aerobic microorganism requiring oxygen to grow and survive, it could multiply under anaerobic conditions as well. Anaerobic oxidation had been demonstrated with *Thiobacillus ferrooxidans* using elemental sulfur with ferric sulfate.

Factors that influence the growth of *Thiobacillus ferrooxidans* are temperature, moisture, pH and nutrient value. As temperatures increase or decrease, the growth rate is adversely affected. The best pH range for *Thiobacillus ferrooxidans* ranges between 6.5 and 7.5. Likewise, optimum concentrations of nitrogen, vitamins and minerals are required for maximum growth rate [27-28].

3.3.2.7. Canola

Canola is a hyper accumulating cultivar of either rapeseed (*Brassica napus* L.) belongs to the family *Brassicacea*. Turnip, rutabaga, cabbage, Brussels sprouts, mustard, and many other vegetables are also related to the two natural canola varieties. Phytoremediation is an emerging technology that employs use of higher plants for cleanup contaminated ecosystems. These plants had acquired different mechanisms for growth in the presence of PTEs concentrations usually considered phytotoxic. Potential for phytoremediation depends upon the interaction among soil, contaminants, microorganisms, and plants. This complex interaction is affected by a variety of factors, such as climatic conditions, soil properties, and site hydro-geology, argues against generalization, and in favor of site-specific phytoremediation practices. Thus, an understanding of the basic plant mechanisms, and the effect of agronomic practices on plant/ soil/ contaminant interactions would allow practitioners to optimize phytoremediation by customizing the process to site specific conditions. The use of plants to extract PTEs from contaminated soils had emerged as a cost-effective, environment-friendly cleanup alternative. Root growth affects the properties of rhizosphere soil and stimulates the growth of a microbial consortium. Rhizosphere microorganisms might interact symbiotically with roots to enhance the potential for PTEs uptake. In addition, some microorganisms might excrete organic compounds which increase bioavailability, and facilitate root absorption of PTES. Soil microorganisms could also directly influence PTEs solubility by altering their chemical properties. For example, a strain of *Pseudomonas maltophilia* was shown to reduce the mobile and toxic Cr^{6+} to nontoxic and to immobile Cr^{3+} and also to minimize environmental mobility of other toxic ions such as Hg^{2}+$Pb^{2+,}$ and Cd^{2+} [31].

3.3.2.8. Black nightshade (Solanum nigrum)

Black nightshade is a fairly common hyperacculator herb or short-lived perennial shrub, always found in most sewged soils and wooded areas, as well as in disturbed habitats. The berry is mostly 6–8 mm diameter, dull black or purple-blacka medicinal weed belonging to the genus *Solanum*. It is considered to be highly toxic to livestock and humans, however, its ripe berries and cooked leaves are used as food in some locales; and plant parts are used as a traditional medicine. Some of the major species within the *Solanum nigrum* complex are: *Solanum nigrum*, *S. americanum*, *S. douglasii*, *S. opacum*, *S. ptychanthum*, *S.retroflexum*, *S. sarrachoides*, *S. scabrum*, and *S. villosum*. The rhizosphere of black nightshade possesses the ability to decontaminate infectious pathogens[32].

3.3.2.9. Corn (Zea mays L)

Corn is a grain domesticated in many countries since prehistoric times and is the most widely grown grain crop in Egypt. Its widely used in the biochemical industry and research as a culture medium to grow many kinds of microorganisms. "Feed Corn" is being used increasingly for heating, specialized Corn stoves are available and use either feed Corn or wood pellets to generate heat. Corn cobs are also used as a biomass fuel source. Corn is relatively cheap and home-heating furnaces had been developed which use Corn kernels as a fuel. Corn is increasingly used as a feedstock for the production of ethanol fuel that is mixed with gasoline to decrease the amount of pollutants emitted when used to fuel motor vehicles. Corn is widely used as a feedstock for biogas plants. Here the Corn is harvested, shredded then placed in silage clamps from which it is fed into the biogas plants. This process makes use of the whole plant rather than simply using the kernels as in the production of fuel ethanol. Some forms of the plant are occasionally grown for ornamental use in the garden. For this purpose, variegated and colored leaf forms as well as those with colorful ears are used. Corn is considered as one of the most well-known hyperaccumulator that absorb high quantities from PTEs when grown in contaminated soils.

Potential toxic elements such as Zn and Cu are essential nutrients for plants and are present in sewage effluent. Research had shown the effects of the application of sewage effluent on Cu and Zn levels in maize. Sewage effluent increased in total Zn concentration without being excessive for human consumption [33].

3.3.2.10. Calcium hypochlorite

Calcium hypochlorite is a yellowish white solid relatively stable compound with a strong smell of chlorine and a formula of Ca $(ClO)_2$ not highly soluble in water and widely used for disinfecting soil and water that eliminates bacteria, algae, slime, fungi and other microorganisms. It is produced in two forms, dry or hydrated, the latter is safer to handle. Calcium hypochlorite reacts with carbon dioxide to form calcium carbonate and release dichlorine monoxide. Calcium hypochlorite is best kept in a cool dry place away from any organic material. It is known to undergo self-heating and rapid decomposition accompanied by the release of toxic chlorine gas. Chlorination is a regularly used method for destruction of infectious pathogenic and other harmful organisms in environment. In disinfection, enough chlorine is added to convene the chlorine demand and provide free chlorine residuals. Chlorine compounds are influential strong oxidizing agents; they are sometimes functional to soil as successful means of destroying enteric pathogens. When calcium hypochlorite is added to soil it forms HOCl and OCl- depending on pH and temperature. Hypochlorous acid and hypochlorite are called free chlorine residuals. The effectual distribution of these two species is very important because the disinfecting efficiency of HOCl is 40 to 80 times that of OCl-. Five factors govern the disinfection efficiency of chlorination, i.e., chlorine concentration, type of chlorine, contact time, temperature and pH. Chlorine might become bound to soil colloids, debris, and other organic materials, once chlorine becomes joined with these materials; it is no longer available for disinfection. Four types of chlorines are commonly used in agriculture, i.e., sodium hypochlorite, calcium hypochlorite, gaseous chlorine and chlorine dioxide. Calcium hypochlorite was chosen in the current experiments as it is a dry form of chlorine and when dissolved in water, it is an effective disinfectant that eliminates infectious parasites. It is much safer to deal with compared to both chlorine gas and sodium hypochlorite and easier to store besides being not corrosive and is less cruel on equipment. OnGreen [34] invented an environment-friendly high-efficiency soil disinfection method, using a disinfection medicament with a final concentration of effective components between 10 and 200 milligram/liter containing hypochlorite. However, it should be well thought-out that both hypochlorite's are classified as Toxicity Category I, the highest level of toxicity. It is tremendously corrosive and could badly injure eyes and skin.

3.3. Setting endpoints*:*

The end points were set (Table 3) within the frame of the results gained from the project experiments as well as related data available in the literature.

Table 3. Endpoints used in the risk assessment for remedial amendments.

Remedial amendment	Endpoints
Bentonite	PTEs and POPs in soil ecosystem Health hazard to farm workers
Rock phosphate	PTEs and POPs in soil ecosystem Soil pH Health hazard to farm workers
Arbiscular mycorrhiza includes (AM)	PTEs uptake by plants
Bacillus megaterium var. phosphaticum	PTEs uptake by plants Soil pH
Thiobacillus thiooxidans	PTEs uptake by plants Soil pH
Thiobacillus ferrooxidans	PTEs uptake by plants Soil pH
Canola hyperaccumulator	Vegetative characters PTEs uptake by plants
Corn hyperaccumulator	Vegetative characters PTEs uptake by plants
Black nightshade hyperaccumulator	Vegetative characters PTEs uptake by plants
Calcium hypochlorite	Dehydrogenase activity in soil Infectious pathogens Health hazard to farm workers

Table 4. Levels of adverse impacts of the proposed remedial amendments.

Remedial amendment	Levels of adverse impacts
Bentonite	Minor, marginal, likely
Rock phosphate	Major, highly likely
Arbiscular mycorrhiza includes (AM)	Minor, marginal, highly unlikely.
Bacillus megaterium var. phosphaticum	Minor, marginal, highly unlikely.
Thiobacillus thiooxidans	Intermediate, likely
Thiobacillus ferrooxidans	Intermediate, likely
Canola hyperaccumulator	Intermediate, likely
Corn hyperaccumulator	Intermediate, likely
Black nightshade hyperaccumulator	Intermediate, likely
Calcium hypochlorite	Major, highly likely

3.3.1. Characterization of adverse impacts was not only limited to identifying hazards of the adverse impacts of the proposed remedial amendments, but extended to link them with the indented goals and endpoints. The levels of adverse impacts and consequences (Table 4) were expressed in terms as major, intermediate, minor, marginal, highly likely, likely, unlikely or highly unlikely.

A remedial amendment impact depends on its concentration and the manner in which it penetrates the ecosystem or living organisms. Some are acutely toxic in small dosages by inhalation.

Some must be ingested to have an effect, while others could be absorbed through the skin. Their extent of contamination and persistence in the ecosystem depends upon soil type, moisture, temperature, ultraviolet ray exposure (sunlight), pH, and soil biomass intensity, as well as on their degradability and original level of usage. In addition, the remedial amendments actually applied to ecosystem might be converted into new compounds of different toxicity as a result of the environmentally or biologically catalyzed reactions. Generally, the nature of these environmental and biological interactions with remedial amendments is poorly understood. However, the biological and physical degradation of parent remedial amendments in the ecosystem could give rise to a variety of new compounds. These new metabolites and degradation products might be of greater, lesser, or equal toxicity, compared to the parent remedial amendment.

3.3.2. Bentnite

- In view of the widespread distribution of bentonite in nature and its vast uses, it was repeatedly confirmed that human or ecosystem exposure to low concentrations of bentonite is ubiquitous. Safety tests conducted showed bentonite as a safe product.
- From an ecological and agronomic point of view bentonite application to the sewaged soil ecosystem might have very limited adverse impacts, if any.
- Abiotic degradation of bentonite into other minerals takes place only on a geological time scale.
- From a health risk point of view there is limited information on occupational exposure to bentonite dust during mixing with soil.
- The highest reported values for total and reparable bentonite dust concentrations were, respectively, 1430 and 34.9 mg/m3, although most values were below 10 mg/m3 for total dust and below 5 mg/m3 for reparable dust. Bentonite-exposed persons are only apt to mild non-specific tissue changes in association with limited interstitial fibrosis [20].
- In some of studies, radiological abnormalities had been reported.
- Bentonite often contain quartz, which is known to cause silicosis and lung cancer.
- Bioaccumulation of bentonite in food-chain appeared minimal, if occurs at all.
- Bentonite appears to be a lung and eye irritant depending on its source, it could also contain toxins [35].
- In some studies, radiological abnormalities had also been reported. There exist no registered cases of marked diffuse/nodular pulmonary tissue fibrotic reaction due to free silica bentonite.
- No quantitative estimates of the potency of bentonite to cause adverse pulmonary effects could derive.
- Bentonite has low toxicity towards aquatic organisms [36].

3.3.3. Rock Phosphate

- Rock phosphate contains a number of potentially harmful elements that might cause some ecological and health adverse consequences.
- From an ecological and agronomic point of view long-term risks associated with rock phosphate application had not been investigated in detail and are largely unknown.
- Fluorine and other PTEs are expected to increase in soils receiving regular applications of rock phosphate [37].
- Rock phosphate lead to a minimal elevation of phosphate in water sources.
- Rock phosphate immobilizes PTEs and hence reduces their solubility in soil and their availability to plant uptake and toxicity to soil biomass [38].
- From a health point of view although no human data on chronic toxicity of rock phosphate were identified in the literature, however, the majority of published data focused on accidental or intentional ingestion of rock phosphate.
- There are insufficient data from human and animal studies to establish a safe upper level for rock phosphate. Few studies had reported diarrhea and mild gastrointestinal symptoms at doses of 750 to 2250 mg/day. The osmotic diarrhea reported in these studies was mild and reversible in nature. In addition, physiological changes in calcium and parathyroid hormone levels had been associated with intakes of 1500 mg/day and above [39].
- There are a limited number of studies on the oral toxicity of rock phosphate.
- Kidney lesions had been reported in rats following exposure to acute doses of rock phosphates (approximately 5000 mg kg^{-1} body weight/day, equivalent to about 1200 mg/kg body weight /day phosphorus).
- Pathological effects in the parathyroid, kidneys and bone had also been reported in sub chronic studies at high doses (approximately 4000 mg/kg body weight /day, equivalent to about 1000 mg/kg body weight /day phosphorus).
- Rock phosphate is often tainted with radionuclides and other radioactive materials as uranium, radium, radioactive lead, radon, thorium and polonium might all be found in rock phosphate. As a result, it might pose a health risk to anyone comes into physical contact with it. Many of these radioactive materials are carcinogenic.
- The high content of PTEs in rock phosphate might pose some health risks. Cd is the most notable, and it might be extremely toxic. Soils fertilized with rock phosphate might contain two to six times the average amount of Cd.

  That might cause lung damage, shortness of breath and persistent coughing, serious internal damage to kidney, liver and gastrointestinal tract. Long-term exposure to Cd might lead to chronic health conditions, such as anemia, pulmonary and kidney dysfunction. Exposure to Cd had also been linked to several types of cancer, including lung and prostate.

- Rock phosphate is often high in fluorides, including fluorosilicic acid, a toxic substance that could pose a serious health risk. Fluoride might be linked to leukemia and lung cancer and might also play a role in osteosarcoma.
- Other toxic PTEs such as Cr, Pb, Mn, B, As, HG and V might be found in rock phosphate, depending on the rock from which it comes. These PTEs could contribute to a huge variety of health problems such as neurological symptoms, organ damage, birth defects and cancer.
- High exposure to rock phosphate might also make people more vulnerable to bone damage [39].

3.3.4. *Thiobacillus sp. and PDB*

- From ecological and agronomic points of view there is no or little information, if any, on any hazards associated with the application of the proposed microbial remedial amendments, *i.e.* AM*, Thiobacillus sp, Bacillus megaterium var. phosphaticum,* as far as they are all indigenous micro-flora isolated from the recipient soil ecosystem.
- Survival of microbial remedial amendments and how they infect a new host needs to be better understood to eliminate or minimize health risks.
- As a rule of thumb, not all the *Thiobacilli* labeled as *Acidithiobacillus* are ubiquitous, and introducing them into soil ecosystems has no adverse impacts.
- As far as human (or animal) pathology goes, these bio-agents could conceivably grow in the gut and might cause some allergic reactions and certain non-oral routes of infection, such as respiration.
- *Thiobacillus* (*Acidithiobacillus*) *ferrooxidans* produce sulfur dioxide which had been documented to cause many symptoms when humans are exposed to them. It has recently been linked as a causative agent in disorders of the gastrointestinal tract, eyes, and respiratory system in humans and animals [27,28].

3.3.5. *Canola (Brassica napus)*

- Data collected on the allergenicity of canola pollen is often confounded by the other plants, particularly grasses, which flower at similar time. People who complained of symptoms in relation to the flowering of canola were rarely allergic to the plant and fewer than half were atopic. Nevertheless, they usually show increased bronchial reactivity during the season, which might be due in some cases to other allergens but in others to non-specific irritant effects of the air. There is no evidence of cross-reactivity between canola and grass pollen.
- Occupational allergies to canola appear as either immediate hypersensitivity or delayed hypersensitivity reactions. The latter frequently occurs as a consequence of handling plant material and generally manifests as contact dermatitis. However studies showed that exposure to canola flour might be a possible cause of occupational asthma in farmers.
- Those at risk from canola pollens include rural population and farm workers in particular. Canola growers might be infected with sclerotia fungi, only at flowering stage because the pathogen uses petals as a food source; certainly no infection occurs prior to flowering [40].
- The health risks are associated with many ecological and agronomic management practices, e.g., crop rotation, less susceptible variety, field history and weather forecasts (for example, rain, high humidity, temperatures).

3.3.6. *Corn*

- Corn contains lipid transfer protein, an indigestible protein that survives cooking. This protein had been linked to a rare and understudied allergy to Corn in humans [41]. The allergic reaction could cause skin rash, swelling or itching of mucous membranes, diarrhea, vomiting, asthma and, in severe cases, anaphylaxis. It is unclear how common this allergy is in the general population.

3.3.7. Black nighshade

- The toxicity of *Solanum nigrum* varies widely depending on the variety, and poisonous plant experts' advice: Unless you are certain that the berries are from an edible strain, leave them alone [42].
- All parts of the plant could be poisonous, containing toxic glycoalkaloids at 0.524% (dry weight). The toxins are most concentrated in the unripe green berries. A glycoalkaloid solanine present in *S. nigrum* and is extremely toxic and potentially fatal. Poisoning symptoms are typically delayed for 6 to 12 hours after ingestion [43]. Initial symptoms of toxicity include fever, sweating, vomiting, abdominal pain,

diarrhea, confusion, and drowsiness. Death from ingesting plant parts results from cardiac arrhythmias and respiratory failure. Children had died after eating unripe berries, and consumption had caused livestock fatalities[44].

- The solanine in *Solanumnigrum* is not destroyed during normal cooking because the decomposition temperature of solanine is about 243 ºC [43].

3.3.8. Calcium hypochlorite

- From an ecological and agronomic point of view, calcium hypochlorite has some temporary adverse impacts on soil biomass.
- Many reactions of calcium hypochlorite might cause high risk of fire and explosion when being on contact with acid(s), combustible substances and/or reducing agents where it releases irritating or toxic fumes (or gases) in a fire.
- Calcium hypochlorite is a strong oxidant and reacts violently with combustible and reducing materials. It reacts violently with ammonia, amines nitrogen compounds and many other substances, causing explosion hazard. It attacks plastics and many PTEs forming flammable/explosive gas (hydrogen).
- Calcium hypochlorite could be absorbed by ingestion and inhalation. A harmful concentration of airborne particles could be reached quickly when dispersed, especially if powdered.
- Calcium hypochlorite is corrosive to eyes, skin and respiratory tract. Inhalation of its decomposition products might cause lung edema, but its effects might be delayed. The symptoms of lung edema often do not become manifest until a few hours had been passed and they are aggravated by physical effort. Immediate administration of an appropriate inhalation therapy by a doctor or a person authorized by him/her should be considered[45].

Understanding the adverse impacts of the remedial amendments allows risk decision-makers to make informed decisions regarding the existence of risk and whether it exceeds acceptable levels. In-effective use or miss-understanding of these impacts could cause serious and un-acceptable risk exposure, subjecting human health and ecosystem to increased risk of irreversible health damage, or even death.

3.4. Risk characterization

Risk characterization culminated all work done through gained results where assessors described and estimated risks posed to ecological entities and human health within their uncertainties context in a frame of transparency, clarity, consistency and reasonableness. Risk characterization well thought-out all predictable adverse risks and the consequences associated with the use of the proposed remedial amendments when interacted with the receiving sewaged soil ecosystems whether being direct or indirect, immediate or delayed, combinatorial or cumulative, predicted or un-predicted, high, medium or low, negligible or indeterminate, acute or chronic, severe or mild, fast or low acting and/or directed to one or many species.

The research team reached two main protocols for the bioremediation of contaminated sewaged soils. The first deals with decontamination of PTEs and POPs, and the second deals with decontamination of infectious pathogens.

The reached sequence protocol included two main phases the first phase lasted for two months through treating the contaminated sewaged soil ecosystem with a conglomerate of chemical and microbial remedial additives’ persuasive enough to remediate PTEs and POPs and the second phase was phytoremediation of the soil ecosystem. The single remediation protocol was disinfecting infectious pathogens in the sewaged soil ecosystem by calcium hypochlorite.

The chemical remedial amendments proposed in the sequence reached protocol comprised of bentonite and rock phosphate. The microbial remedial amendments proposed in the first reached protocol comprised of arbuscular mycorrhiza (AM), *Bacillus megaterium var. phosphaticum*, *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*. The phytoremediation was done during winter season with canola or black nightshade hyperaccumulator and during summer season with corn hyperaccumulator. In the second reached protocol calcium hypochlorite was used to decontaminate infectious pathogens in sewaged soil ecosystem. The expected adverse ecological and health impacts of all the remedial amendments used in both protocols are described above in details.

The first sequence reached protocol was applied at two stages. The first one extended for two months and included supplementing and fortifying the sewaged soil ecosystem with the chemical and microbial amendments. During these two months the microbial amendments *Bacillus megaterium var. phosphaticum*, *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* grew in the sewaged soil ecosystem and intensified their population before start acting in the bioremediation process.

Bacillus megaterium var. phosphaticum acted on transferring tri-calcium phosphate in the soil ecosystem, whether native or added with rock phosphate, to mono- and di-calcium phosphate that distorted the balance between PTEs and phosphate rendering the formers more pertinent to AM action and hence increasing their uptake by the hyper accumulating plants. As mentioned above, this biological process is well thought-out as a normal bioactivity permanently going on in most cultivated soil ecosystems. The bioremediation process implemented in the current work is no more than enhancing this natural biological process through fortifying the sewaged soil ecosystem with a microbial agent that is capable to achieve it at a higher potency. This process does not bear any adverse ecological or impacts. Also nothing mentioned in literature that *Bacillus megaterium var. phosphaticum* has any infectious characteristics towards humans or livestock. Hence the risks related with *Bacillus megaterium var. phosphaticum* might be considered acceptable. The expected adverse risks associated with the use of *Bacillus megaterium var. phosphaticum*, if any, when interacted with the receiving sewaged soil ecosystems are considered indirect, delayed, cumulative, predicted, indeterminate, mild, slow acting and directed to many species.

Soil fortification with *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* intended to lower the pH of the recipient sewaged soil ecosystem through oxidation of mineral elemental sulpher forming sulphuric acid. This is a natural biological process occurring forever and a day in all cultivated soil ecosystem. The bioremediation process implemented in the current work is no more than enhancing this natural biological process through fortifying sewaged soil ecosystem with both *Thiobacillus* sp. that can perform it at a more effectual rate. This process does not bear any adverse ecological impacts. Also nothing mention in literature that *Thiobacillus sp.* had any infectious characteristics towards humans or livestock. Hence the risks associated with *Thiobacillus sp.* are acceptable. The expected adverse risks associated with *Thiobacillus* sp. use, if any, when interacted with the receiving sewaged soil ecosystems are considered indirect, delayed, cumulative, predicted, indeterminate, mild, low acting and directed to many species.

Soil inoculation with AM that has a distinct advantageous action with higher plants was done to increase the surface absorption area of plant roots to guarantee maximum nutrient uptake by plants for the most part from the targeted PTEs. This is a natural biological process taking place for all time in all cultivated soil ecosystems. The bioremediation process implemented in the current work is no more than enhancing this natural biological process through fortifying the sewaged soil ecosystem with AM spores that can perform nutrient uptake at an elevated effective function. This biological process does not bear any adverse ecological impacts. Also nothing mentioned in literature that AM has any infectious characteristics towards humans or livestock. Hence the risks associated with AM inoculation are acceptable. The expected adverse risks associated with AM use, if any, when interacted with the receiving sewaged soil ecosystems are considered direct, delayed, cumulative, predicted, indeterminate, mild, low acting and directed to many species.

Soil treatment with bentonite aimed to increase the adsorptive power of the sewaged soil ecosystem. The application of bentonite seems to have some ecological and health adverse impacts as mentioned before. Bentonite contains some PTEs and some radioactive sources that might faultily adverse soil ecosystem. Parallel to that it might cause some health impacts to those who came in close contact with this amendment. The bioremediation process implemented in the current work raised bentonite content in the soil ecosystem, however being not that much to put forth severe adverse impacts. However precautious and imitative measures have to be cared about on the basis of the periodical monitoring results of the sewaged soil ecosystem.

Despite of that, the risks associated with bentonite application are acceptable. The expected adverse risks associated with bentonite use, when interacted with the receiving sewaged soil ecosystem are considered direct, immediate, cumulative, predicted, medium, sever, fast acting and directed to many species.

Rock phosphate was applied to increase the phosphate content in the soil ecosystem on the whole when reacted with *Bacillus megaterium var. phosphaticum*. The higher the content of available phosphate in the sewaged soil ecosystem the higher transform in PTEs availability. The application of rock phosphate seemed to have some ecological and health adverse impacts as mentioned before. Rock phosphate contains some PTEs and some radioactive materials that might roughly adverse soil ecosystem. Parallel to that it might cause some health impacts to those who came in close contact with this amendment. The bioremediation process implemented in the current work raised its content in the soil ecosystem, however being not that much to exert severe adverse impacts. However precautious and mitigation measures must be cared about on the basis of the periodical monitoring results of the sewaged soil ecosystem. Despite of that, the risks associated with rock phosphate application are acceptable. The expected adverse risks associated with rock phosphate use, when interacted with the receiving sewaged soil ecosystems are considered direct, immediate, cumulative, predicted, high, indeterminate, severe, low acting and directed to many species.

Phytoremediation of the sewaged soil ecosystem with canola during summer season or Corn or back nightshade during winter season was made to get rid of PTEs and POPs from the contaminated sewaged soils after two month of treating with both chemical and microbial amendments. The cultivation of canola plant seemed to have some health adverse impacts to those who came in close contact with it as mentioned before.

The phytoremediation process implemented in the current work abridged markedly the content of PTEs in soil ecosystem, and does not exert any severe adverse impacts. However precautious and mitigation measures must be cared about on the basis of the periodical monitoring results of the sewaged soil ecosystem. Despite of that, the risks associated with canola, Corn or black nightshade cultivation are acceptable. The expected adverse risks associated with their cultivation, when interacted with the receiving sewaged soil ecosystems are considered indirect, delayed, combinatorial, predicted, medium, indeterminate, mild, fast or acting.

In the second reached protocol the use of calcium hypochlorite to decontaminate infectious pathogens has some definite ecological and health adverse impacts as described above. The soil biota is expected to be adversely impacted with the application of calcium hypochlorite to soil ecosystem. Although the dehydrogenase activity was decreased after the application of calcium hypochlorite, it restored their normal level after one week, because calcium hypochlorite is a sublimated compound. Varied adverse health impacts to human and livestock are probable from the misuse of calcium hypochlorite. However precautious and mitigation measures must be cared about on the basis of the periodical monitoring results of the sewaged soil ecosystem and health status of those who came in contact with calcium hypochlorite. Despite of that, the risks associated with calcium hypochlorite are acceptable as far as precautious measures are well applied. The expected adverse risks associated with calcium hypochlorite, when interacted with the receiving sewaged soil ecosystems and health status of those who came in contact with this amendment are considered direct, immediate, cumulative, high, indeterminate, acute, severe, fast acting and directed to one or many species.

3.5. Mitigation and precocious measures

Mitigation and precocious measures included whether or not risks are accepted, and setting mitigation and precocious measures (Table 5) to combat the adverse impacts of the proposed remedial amendments through avoidance, additional measures and/or contingency plan**.** Avoidance strategy was recommended when the risk factor does not pose any considerable threat, i.e., ignoring the risk, and doing nothing and accepting the consequences. Additional measures were only filled for those risks for which control mitigation strategy is decided. A contingency plan was proposed for high impact risks with a high probability of occurrence, just in case the basic measures fail to perform. Generally, a periodical monitoring should be followed to assess the adverse impact.

Table 5. Mitigative and precocious measures towards the proposed remedial -amendments (Rock phosphate, canola, corn, nightshade and Calcium hypochlorite).

Remedial amendment	Risk acceptability	Mitigative strategy	Mitigative and precocious measures
			Rock phosphate
Rock phosphate	Accepted	Additional measures	Risk reduction of the ecological and agronomic adverse impacts might primarily be achieved by transforming labile content of PTEs in rock phosphate to relatively stable forms. Phosphate-immobilized PTEs are chemically and biologically stable in the sewaged soil ecosystem. This could be done by eventual organic manuring widening C/N in soil. The potential of soil PTEs contributing to surface or ground water will be minimized. From a health point of view, a supplemental intake of 250 mgiclay would he expected not to produce adverse effects, including mild gastrointestinal upset. This is equivalent to 4.2 mg/kg bw in a 6.0 kg adult. Assuming a maximum intake of 2100 nigida.y, an estimated total intake of 2400 mg/day (40 mg/kg bwiday in a 00 kg adutt) wpuld not be expected to result in any adverse impacts. A periodical monitoring should be followed to assess the adverse impact.
			Canola
Canola hyperaccumulator	Accepted	Avoidance	Precocious measures must be exercised to avoid being in touch with canola pollens during the flowering stage. The risk of fire during: drying canola might be reduced by cleaning the seed to remove light or fine material before drying, removing accumulations of debris from the walls and other areas of the dryer and using wind dleflectors to prevent drawing airborne material through the burner, and avoiding over drying the seed. Frequently check the dryer and periodically clean it to reduce the fire hazard.
			Corn and Black nightshade
Corn hyperaccumulator	Accepted	Avoidance	No ecological, agronomic or serious health adver5e impacts concerning growing Corn were neither noticed within our experiment nor stated in literature.
Black nightshade hyperaccumulator	Accepted with precocious measurers	Contingency plan	It is strictly prohibited to eat any organ of back nightshade at any growth stage.
			Calcium hypochlorite

Calcium hypochlorite	Acceptedwith	Contingency plan	Personal protection precocious measures must be extensively cared about when coming in contact with calcium hypochlorite such as using face shield, fitter respirator, protective clothes, safety glasses and chemical-resistant gloves. Always sweep spilled substance into air tight, dry containers, and then remove to safe place. Avoid contacting calcium hypochlorite with combustibles and reducing agents. Store calcium hypochlorite in awe!l closed container far away from food and feed stuffs. Calcium hypo chlorite might react Mth some organic ingredients in the soil ecosystem forming toxic compounds such as trihalomethanes, dioxins and polychlorinated biphenyls (PCBs). Medical care should always be available.

4. Conclusions

Risk assessment is an ordered practice conducted in a scientifically sound and transparent approach on a case-by-case basis in relation to the likely prospective of the receiving ecosystem. It is designed to identify whether an adverse impact exists, and if so, to gather information on its nature and severity. The adverse impact should be identified to determine its relevance ecological, agronomic and/or health impacts. In the current work, risk assessment cared about all consequences derived from the proposed remedial amendments was conducted. Scientific data for risk assessment were generally collected from a variety of sources, such as literature, technical information, results gained from the project experiments, independent scientists and other interested parties. The key steps of the risk assessment process were to identify and evaluate the potential adverse impacts and to set strategies to mitigate them taking into account uncertainty regarding the level of risk.

Remediation risk assessments were conducted to determine how much of one or more of the proposed remedial amendments must be removed or mitigated to achieve acceptable risk levels sufficient to protect human health and ecosystem.

Based on the results of ecological and health assessments, restoration recreate to some extent the structure and function of a sewaged soil ecosystem after bioremediation. In case, ecological restoration was performed after remediation, risks associated with this restoration must be well thought-out.

As scientific truth is always somewhat uncertain, and information is characterized by kinds and degrees of doubt, uncertainties interfere causing what occurs to differ from what was expected. Ecological and health uncertainty is of two basic kinds, what is not known at all, and errors in what is known. The latter type is a quantitative departure from the truth, and could sometimes be expressed statistically as a distribution of a number of repeated measurements around a mean value. Other forms of quantitative uncertainty are incomplete data which is not gathered with a statistical design, inappropriate extrapolation, and temporal and spatial variability of the measured parameters. Most important ecological problems, however, suffer from true uncertainty, that is, indeterminacy, or events with an unknown probability.

Monitoring is always applied as a tool to detect unexpected and long-term ecological impacts. It could also be a means to reduce uncertainty, address assumptions made during the risk assessment and to validate its conclusions on a wider level of application and to establish a causal link or pathway between amendments and adverse impacts. In monitoring, established criteria and thresholds for the acceptable/ unacceptable levels of risk including those set out in national legislation or guidelines, as well as the protection goals as identified when setting the context and scope for the risk assessment, any relevant experience with the proposed remedial amendments and practices associated with its use in the likely potential receiving sewaged soil ecosystem, scientific benefit analyses carried out using similar principles of sound-science, ability to identify, evaluate and confine adverse impacts as well as to take appropriate response measures of the overall uncertainty must be cared about.

The precautionary approach of the reached protocols necessitated ensuring an adequate level of protection ensuring safety during handling and use of the varied input amendments that might have some adverse ecosystemal, agronomic and/or health impacts. In this respect health risks concerning farm workers and product consumers are of utmost significance.

Worldwide, regular use of ecological and health risk assessments has supported safe application of remedial amendments in sewaged soil ecosystems. A full ecological and health risk assessment for all proposed amendments should always consider accessible contamination, remedial materials, residual contamination and consequent land uses.

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