



Review

Selenium and Nano-Selenium Biofortification for Human Health: Opportunities and Challenges

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Abstract: Selenium is an essential micronutrient required for the health of humans and lower plants, but its importance for higher plants is still being investigated. The biological functions of Se related to human health revolve around its presence in 25 known selenoproteins (e.g., selenocysteine or the 21st amino acid). Humans may receive their required Se through plant uptake of soil Se, foods enriched in Se, or Se dietary supplements. Selenium nanoparticles (Se-NPs) have been applied to biofortified foods and feeds. Due to low toxicity and high efficiency, Se-NPs are used in applications such as cancer therapy and nano-medicines. Selenium and nano-selenium may be able to support and enhance the productivity of cultivated plants and animals under stressful conditions because they are antimicrobial and anti-carcinogenic agents, with antioxidant capacity and immune-modulatory efficacy. Thus, nano-selenium could be inserted in the feeds of fish and livestock to improvise stress resilience and productivity. This review offers new insights in Se and Se-NPs biofortification for edible plants and farm animals under stressful environments. Further, extensive research on Se-NPs is required to identify possible adverse effects on humans and their cytotoxicity.

Keywords: human disease; cereal crops; vegetable crops; hyper-accumulators; biofortified crops

1. Introduction

The discovery of selenium in 1817 triggered a huge amount of innovative scientific inquiry into human health. Selenium is an essential micronutrient for humans and animals as well as some lower plants, but it still needs more investigation to establish whether or not it is essential for higher plants [1,2]. Selenium (Se) plays a vital role in the metabolism of humans, animals, and many prokaryotes as well as some algae [1]. This micronutrient is the only metalloid that is incorporated into specific proteins, called “selenoproteins”, and genetically encoded as well as forming a constitutive part of selenocysteine (SeCys), “the 21st amino acid” [3]. In total, 25 selenoproteins have been identified in the human proteome and are often oxido-reductases, including SeCys as a catalytic residue [4,5]. These selenoproteins mainly have wide redox functions that are vital for regulating human immunity [6], mediating thyroid disorders [7], and for the health of the reproductive system [1,3,8,9]. The essential role of selenium in human health has been confirmed by several researchers [10–16]. A major distinguishing feature of Se is the narrow margin between Se-deficiency ($<40 \mu\text{g day}^{-1}$) and toxicity ($>400 \mu\text{g day}^{-1}$) [17]. The recommended daily dose for adults is $55 \mu\text{g day}^{-1}$ in the USA and 55 to $70 \mu\text{g day}^{-1}$ in Europe [18]. Selenium is called the “the essential poison” and characterized as “the double-edged sword” due to its biological effects under deficiency and toxicity [13].

Selenium and nano-selenium (nano-Se) or (Se-NPs) have been used in the maintenance of human health [19]. They can be applied in biomedical and drug delivery [20] dietary supplements, therapeutic agents [18], and nano-medicine applications [21]. The antimicrobial and anticancer properties of both Se and Se-NPs have been confirmed [19,22]. The biofortification of edible foods [23,24] and feeds [25,26] with Se and Se-NPs is an important approach to support human and livestock health.

The primary natural source of Se in foods is crop uptake from soil [13,27]. There are wide geographic variations in the Se content of soils, meaning some regions face Se deficiencies while others have Se toxicity issues based on the Se content in their crops, with both situations having negative impacts on human health [28]. Crops grown in Se deficient soils can be biofortified, including the use of both soil-based and foliar fertilizers to correct the deficiency [27]. Food crops that are commonly biofortified include cereals [29], leafy vegetables like spinach [30] and lettuce [31], and fruits like strawberry [24,32,33] and pomegranate [23]. Due to their lower toxicity, strong capacity to scavenge free radicals, higher bioavailability, and stimulation effect, Se-NPs have been recently used in the production of plants [23,24,34–36], fish [37–40], livestock [41], and poultry [42–48].

Therefore, this review explored available information on the use of Se and Se-nanoparticles in biofortification. The use of selenium and nano-selenium to promote human health is discussed, including the biofortification of crops through soil and other amendments. We also investigated the role of Se and nano-Se to support crops under stress.

2. Selenium and Nano Selenium: General Information

Although Selenium and its nanoparticles share some common and general properties, they important differences based on their unique chemical, physical, and biological properties (Table 1). For example, bulk elemental Se is not water soluble, but Se-NPs are partially water soluble (Figure 1). The behavior and biological features of Se and Se-NPs in the nutrition of higher plants and humans may differ. The role of Se in human nutrition has been confirmed, whereas the biological effects, recommended daily intake and toxicity/deficiency levels of Se-NPs still need more investigations [3,8,10,11,19,49]. Indications of the general role of Se-NPs on human nutrition have been distinguished through studies on fertilization of crops [50–53], poultry [42–44,46–48,54], and human health [18,19,21,55–57].

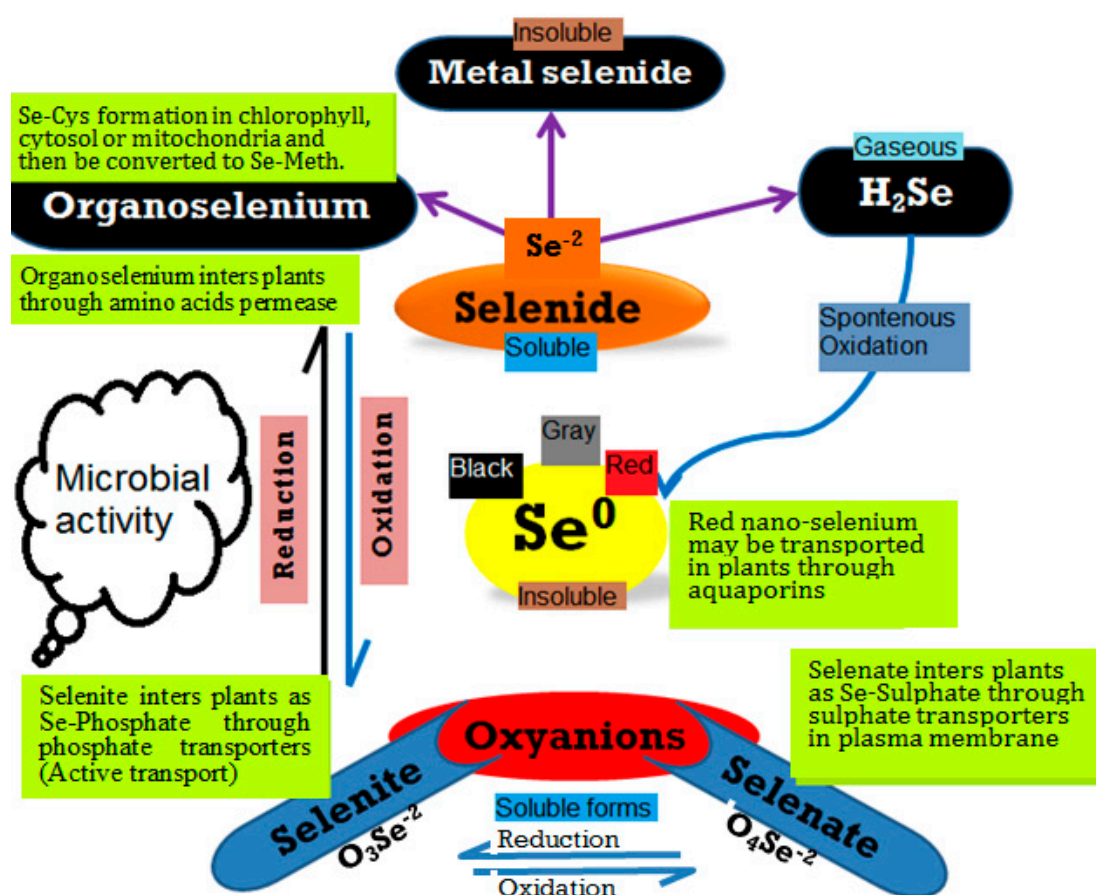


Figure 1. An overview of selenium and its transformations in the soil environment. Different pathways for the fate and transformation of Se and its forms in the environment can be distinguished, including selenate, selenite, and elemental nano-Se.

There are many studies on Se and Se-NPs concerning their potential impact on human health, but the situation is different for higher plants, where much effort is still needed. Uptake from the soil and translocation as well as transformation of Se-NPs in higher plants needs more research. Will these nanoparticles be transformed into toxic forms? What will happen if Se nanoparticles are added or co-applied with another nanoparticle? What are the conditions that control Se-NPs transformations in the rhizosphere? What are the expected ecotoxicological effects of applied Se-NPs in the rhizosphere? At present, there are limited studies of the role of Se-NPs in plant nutrition [23,24,58–60], but the biogeochemistry of Se and Se-NPs in agroecosystems and their speciation in cultivated plants are important issues in terms of biofortification of crops for human health [13,61].

Major questions exist regarding Se and Se-NPs biofortification. Do biological Se-NPs have the ability to replace mineral Se-fertilizer in the framework of sustainable agriculture [62,63]? Will it be possible to find standard levels for deficiency and toxicity of Se-NPs as has been done for Se for humans, animals and higher plants? It is important to understand the different forms of Se, including inorganic (i.e., selenate, selenite, selenide, and elemental nano-Se) and organic (i.e., selenomethionine and selenocysteine), as these are important for Se behavior, especially in soil environments (Figure 1). These forms might control Se availability for plant uptake with contributions to the biofortification process [64–66].

Table 1. The biological features of selenium and nano-selenium and the possible roles in plant and human nutrition.

Comparison Item	Selenium (Se)	Selenium Nanoparticles (Se-NPs)
Plant Nutrition		
Essentiality	NOT yet confirmed, but it is a beneficial nutrient at low concentrations [1]	NOT yet confirmed, but may have a positive impact on levels of bio-compounds beneficial for human health in treated plants [60]
Main uptake form	Selenate (SeO_4^{2-}) through sulfate transporter (e.g., SULTR1;1 and SULTR1;2), selenite (SeO_3^{2-}) via phosphate transport (like OsPT2), and silicon transporter (OsNIP2;1) in roots [67]	Unclear (may be through a passive diffusion process), Se-NPs are soluble, highly stable, have low toxicity, and high bioavailability [23]
Converted form after uptake	Uptake is only by roots, both selenate and selenite will be converted into organic forms like SeCys, SeMet, and MeSeCys [68]. SeMet and MeSeCys are the most dominant species in Se-enriched plants	There is bioavailability of Se-NPs in plants, Se-NPs uptake could occur by roots, then transform into organic Se compounds like SeCys, SeMet, and MeSeCys, with dominance of SeMet [68]
Translocation from roots to shoots	Chemical Se-NPs and selenite have similar translocation of Se from roots to shoots during the longer exposure period (72 h), whereas biological Se-NPs rarely translocate to shoots [68]	A few Se-NPs may transport from roots to shoots due to their rapid assimilation into selenite and organic forms [68]
Main functions in plant	Selenium may increase plant growth and biomass; protect plants from abiotic/biotic stresses; deter herbivores via volatile Se (dimethyl selenide) [69]	Se-NPs (especially 5–200 nm), increase activities of some enzymes like GSH-Px, TrxR, and GST could scavenge free radicals, have excellent bio-availability, low toxicity, and high biological activity in plants [23]
Toxicity level	For agricultural crops $< 50 \text{ mg Se kg}^{-1}$ [23], for most angiosperm species $> 10\text{--}100 \text{ mg Se kg}^{-1} \text{ DM}$ [70]	About 100 mg kg^{-1} is not toxic for most cultivated crops [71], 275 mg L^{-1} is the toxic level for sorghum [34]
Deficiency level	Se content ($\mu\text{g kg}^{-1}$) < 20 for severely deficient areas and $30\text{--}50$ for deficient areas [72]	NOT yet reported
Selenium as a contaminant for plants	At concentrations $> 10 \text{ mg kg}^{-1}$ soil, Se may cause oxidative stress for plants [73]	Few publications addressed Se-NPs as a contaminant [74]. SeNPs can remove Hg in soil [69]
Human Nutrition		
Essentiality and absorption	Confirmed forms of soluble selenium are mainly absorbed in lower part of the small intestine [18]	May be essential. Se-NPs may absorb and be metabolized in the gastrointestinal tract [18]
Main dietary sources	Cereals or grains, poultry, breads, fish, eggs, meat, nuts, and broccoli [10,75]	SeNPs could be used as dietary supplementation due to their therapeutic properties, such as being an anti-carcinogen [18]
Main applications or uses	Biomedical and drug delivery [20], biofortification of edible crops, and animals for human health [76]	Therapeutic or nanomedicine applications [21,56]
Main Se-forms for human intake	Se-methionine, Se-cysteine, and Se-methyl-selenocysteine [77]	Se-NPs in biological or chemical form could be used in nutritional supplements [18] or to combat cancer [57]
Main components in humans	Selenoproteins or the 21st protein-ogenic amino acid selenocysteine, e.g., glutathione peroxidases [16]	NOT yet established
Main functions in humans	Regulates the immune system, mediates thyroid disorders and the health of the human reproductive system [1,9]	Se-NPs may have higher antioxidative capacity compared to other Se-forms (inorganic or organic) and be a more effective therapeutic agent against MeHg neurotoxicity than other forms of Se [78]
Toxicity level	The upper intake level may be more than $400 \mu\text{g day}^{-1}$ [53], mortality results from 1 to $100 \text{ mg Se kg}^{-1}$ body weight [79]	NOT yet established
Toxicity symptoms	The symptoms of mild selenosis (excessive dietary Se intakes) in humans include cracking of nails, hair loss, and dermatitis, while severe selenosis may cause renal failure, acute respiratory distress, and myocardial infarction [70]	NOT yet established. SeNPs have a lower toxicity compared to other forms of Se like selenite or selenomethionine [18]
Deficiency level	Less than $40 \mu\text{g day}^{-1}$ or less than $11 \mu\text{g day}^{-1}$ like in the Keshan region, China causes Keshan disease [53]	NOT yet established. SeNPs are higher in bioavailability efficacy compared to other Se-forms [18]
Deficiency symptoms	Se deficiency may cause several diseases like cardiovascular disease, male infertility, weakened immune system, hypothyroidism, cognitive decline and increased incidence of various cancers [70,80]	NOT yet established
Recommended daily intake	About $55 \mu\text{g day}^{-1}$ based on USDA [1]	NOT yet established
Dietary Reference Intake (DRI)	$100 \mu\text{g Se day}^{-1}$ [77]	NOT yet established

Abbreviations: selenocysteine: SeCys; Se-methyl-selenocysteine: MeSeCys; seleno-methionine: SeMet; glutathione peroxidase: GSH-Px; thioredoxin reductase: TrxR; glutathione S-transferase: GST; DM: dry matter.

The biofortification of cultivated crops using nano-Se may be an important strategy [30] that could be adapted to minimize environmental problems, in particular problems that resulted from the over-use of mineral fertilizers. This is particularly true because Se is rare in the Earth's crust. Nano-Se and Se biofortified edible crops still need more research from different points of view, such as environmental, economic, human health, and animal health perspectives [81–83]. Nano-Se has the potential to protect animals against oxidative stress [84], ameliorate heavy metal stress [85], or function as an effective cancer therapy [86]. The use of nano-Se or Se to support cultivated plants under different stresses is an important strategy due to the ameliorative effects of Se and nano-Se in enhancing the productivity of cultivated crops under harsh conditions such as heat stress [34], nitrate stress [87], pathogen (like *Alternaria solani*) stress [59], NaCl stress [60], and soil salinity stress [24].

3. Biofortification of Selenium and Nano-Selenium for Human Health

Realization of the relationship between Se as a nutrient and human health started with the discovery of the essentiality of this micronutrient in 1817. Many studies have confirmed that Se is essential for human health due to its role in preventing many chronic diseases such as cancer, neurodegenerative diseases, and cardiovascular disease as an essential component of more than 25 enzymes in humans [53]. The level of Se or nano-Se can be increased in foods through the biofortification approach [29,36,52,88–92]. Products from farm animals can also be enriched with Se [41,45,93]. The biofortification of cereal crops including wheat, rice and maize as well as some main vegetable crops including tomato, potato and lettuce will be reviewed in this section.

3.1. Biofortification of Cereal Crops: Wheat, Rice and Maize

The biofortification process is a method in which selected nutrients (e.g., Ca, Cu, Fe, I, Se, and Zn) or nutritional materials are inserted into the food chain [94]. These materials might include folate [95–97], riboflavin [98,99], lysine [100–102], and pro-vitamin A [103]. This can be achieved using the agronomic approach, traditional breeding, and transgenic approaches to reduce nutrient deficiencies for humans [104,105]. The most important nutrients that have been investigated in several biofortification studies include calcium [106], iron [92,107], copper [108], zinc [109–111], iodine [29,92], potassium [112], and selenium [66,113]. The use of Se fertilizers is one of the most common methods for Se-biofortification of several crops [105], such as rice [113–115], maize [116,117], wheat [92,111,118], cowpea [119], potato [85,120], carrot [90,121], turnip [122,123], shallot [124], beans [125], lettuce [91,126], basil [127], strawberry [32,33], and apple [128,129]. Edible plants that have been biofortified with Se [105] or livestock fed selenium-enriched alfalfa [25,130] are used to support human health as reported by the Finnish experience in biofortification with Se through fertilizers. This Finnish experience started in 1984, when the Finnish authorities decided to improve the Se content of foods and feeds by applying synthetic fertilizers containing Na₂SeO₄. The applied doses of Se to soil reached 10 mg ha^{−1} in 2012 with an optimal level of 70–80 µg in the daily Se intake of the Finnish people.

Malnutrition and micronutrient deficiencies have become a global issue and improving the nutrition of millions of people around the world may be achieved using staple crops and appropriate agronomic practices [105,131]. In the past biofortification mainly involved the main cereal crops (e.g., rice, maize, and wheat) and then moved to include pulse crops as well as some animal-based foods such as milk and cheese [132], meat [133], and eggs [134]. The Se-biofortification of cereal crops depends on Se forms, method of application, the efficacy of Se-fertilizers [118], the time of application, and plant growth stage [83,135]. It also depends on soil properties, in particular soil pH, salinity content, redox potential, organic matter content, and the soil microbial community [13,69,116,136–139]. The Se-biofortification of cereal crops including wheat, rice, and maize could be evaluated under different applied Se-forms and different growth conditions (Table 2). A review of the literature led to the following conclusions:

1. The main Se-forms applied to cereal crops for biofortification include selenate, selenite, selenomethionine (SeMet), methio-seleno-cysteine (MeSeCys), and nano-Se.

- The recommended Se-dose for biofortification of cereal crops mainly depends on the plant species and its variety or cultivar, the application method (seed coating and priming, foliar, or soil application), the growth media (e.g., soil, hydroponics, artificial growth media), the growth conditions (open field, controlled greenhouse, or in vitro experiment), the Se-form (inorganic, organic, or nano form), nano-Se characterization (the method of preparing, the size and color of nanoparticles), the background Se content in the soil, and the agricultural management practices [69,85].
- For wheat crops, the recommended Se-dose under field experiments was 21 g Se ha⁻¹ as a foliar application [89], while an applied dose of up to 120 g Se ha⁻¹ did not cause any visible phytotoxicity symptoms [140]. Under pot experiments, an applied Se-dose of 2.5 mg Se kg⁻¹ soil was a suitable dose for Se-fortification of grain wheat [136].
- For rice crops, Se-foliar fertilization up to 100 g Se ha⁻¹ as sodium selenite produces safe and high converting levels of Se into general rice proteins under field experiments when there was an initial low total soil Se content up to 0.1 mg Se kg⁻¹ soil [141]. The best method to fortify the rice plants was to use 6 mg Se L⁻¹ under NaCl stress as a combination of foliar spraying and seed priming [73]. The recommended applied Se-dose for the growth of rice clearly depends on the growth stage (the seedling, tillering, booting, full heading, and mature stage). Foliar application of sodium selenite (10 mg L⁻¹) at the booting and full heading stages enhanced the accumulation of SeMet, confirming that the previous Se rate is the ideal level for Se-biofortification of rice [115].
- For maize crops, biofortification with Se could be achieved under field conditions through a fertigation system at an application rate of 100–200 g of Se ha⁻¹ as sodium selenite. The applied Se might enhance the nutraceutical value and antioxidant content of maize grains without any leaching of Se into groundwater [142,143]. Ngigi et al. [125] reported that the Se biofortification level (0.3 mg kg⁻¹) could be achieved in three field locations in Kenya using a foliar Se-dose of 20 g ha⁻¹ as sodium selenate, whereas Wang et al. [64] indicated that the Se-level may be up to 30 g Se ha⁻¹ in China.

Table 2. Selenium biofortification results of some selected cereal crops (wheat, rice, and maize) under different growth conditions.

Plant Cultivar (Country, Reference)	Selenium Forms and Added Rate	Experimental Conditions and Se-Biofortified Dose
I. Wheat plants (<i>Triticum aestivum</i> L.)		
Variety BRS 264 (Brazil, [89])	Foliar application of sodium selenate doses: 12, 21, 38, 68 and 120 g ha ⁻¹ at vegetative growth and grain filling stage	Field experiment used soil (pH 5.1; clayey, total Se < 0.018 mg kg ⁻¹), the dose 21 g of Se ha ⁻¹ showed the highest grain Se absorption efficiency and highest grain yield
Cultivar: Gazul during the period from 2001–2011 (Spain, [77])	Survey of the total mean Se in soil (159 µg Se kg ⁻¹) and mean Se in harvested grain (41.3–18.4 µg Se kg ⁻¹)	Field experiment used soil (pH 7.7, clay 70%), accumulation of Se in grain was directly related to N-accumulation in wheat
12 Brazilian cultivars (Brazil, [88])	Sodium selenate, i.e., Na ₂ SeO ₄ added at 13 µM L ⁻¹ Se	Pot experiment seeds were sown for 132 days, the dosage (13 µM L ⁻¹ Se) improved the nutritional value and sulfur content of different cultivars of wheat
Seeds of winter wheat: Xiaoyan No. 22 (China, [136])	Separate treatments of sodium selenite and selenate: 0.5, 1, 2.5, 5, and 10 mg Se kg ⁻¹	Pots filled with soil (Silt 57.8%; pH 7.75 and the total Se 0.078 mg kg ⁻¹), a dose of 2.5 mg Se kg ⁻¹ soil was suitable for fortification
Four Italian durum wheat varieties (Italy, [140])	Foliar-Se applied at rates of 1, 5, 10, 15, 20, 25, 50, 80, 100, and 120 g Se ha ⁻¹ as sodium selenate, Se applied at early stem elongation and at the booting stage	Field experiment, soil pH 7.8, the background total Se-content was 0.130 mg kg ⁻¹ soil, no visible phytotoxicity symptoms were observed even at 120 g Se ha ⁻¹ , which may be the best for fortification of wheat
Variety: Seher 2006 (Pakistan, [144])	Two doses at 300 µM sodium selenate (3 mg Se kg ⁻¹ of soil) was given to the plants, which were harvested after 18 weeks	Natural field soil in pots, two Se- doses were given to plants: one-week post-germination and at the reproductive phase, wheat could be fortified at lower Se levels like in this study

Table 2. Cont.

Plant Cultivar (Country, Reference)	Selenium Forms and Added Rate	Experimental Conditions and Se-Biofortified Dose
II. Rice plants (<i>Oryza sativa</i> L.)		
Cultivar: Xiushui 134 (China, [83])	In hydroponics, foliar and root dressing using selenite, selenate and MeSeCys, soil culture using foliar method (100 μM Se)	Plastic containers used in 2 different experiments, i.e., soil culture and hydroponics, root dressing of selenite caused highest accumulation of organic Se compounds, which are desirable for human health
Rice seeds of Xinongyou No. 1 (China, [78])	Se was added at 50 $\mu\text{g L}^{-1}$ as $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$, after 15 days, Se added to rice seedlings and harvested after 48 h	Pot experiment using a nutrient solution, low added phosphorus (1.5 mM L^{-1}) may promote Se content in rice grains
Se-free white rice lines (China, [115])	Foliar sodium selenite at a rate of 10 mg L^{-1} , at booting and full heading stage of Se-free white rice	Pot experiment filled with soil (total Se: 0.35 mg kg^{-1} DW), foliar sodium selenite fertilizer enhanced the accumulation of SeMet confirming that the utilized Se rate was effective for Se-biofortification
Cultivar: Nipponbare; GSOR-100 (Belgium, [73])	Exogenous applied Na_2SeO_4 as foliar (2, 4, 6, 8, 10, and 12 mg L^{-1}), seed priming (6 mg L^{-1}) and combination of seed priming and foliar spraying	Seedlings sown in PVC tubes that contained 100 g sand and polymer mixture, combination of foliar spray and seed priming was the best method to fortify the rice plants (at 6 mg L^{-1}) under NaCl stress
Cultivar: Selenio (Milano, Italy, [114])	Sodium selenite and selenate solutions at 15, 45, 135, and 405 mg Se L^{-1} harvested 10 days after sowing	Grains sown in plastic trays and incubated in a growth chamber, sprouts fortified by 45 mg L^{-1} contained high Se and phenolic acid yield
Two cultivars: Fengbazhan and Hefengzhan (China, [145])	Soil mixed with sodium selenite at 0.5, 1, and 5 mg Se kg^{-1} soil, plants harvested and grains were collected for analysis	Pot experiment filled with soil, total plant Se was 0.45 mg kg^{-1} DW, pH 5.40, the highest content of SeMet was recorded for 5 mg kg^{-1} in rice grains
Rice seeds: Zhuliangyou 819 (China, [141])	Foliar application of sodium selenite at 25, 50, 75, and 100 g Se ha^{-1}	Field experiment, total soil Se content was 0.1 mg kg^{-1} of soil, Se-foliar fertilization up to 100 g Se ha^{-1} produced a safe and high conversion of Se into general rice proteins
Brown rice, cultivar: Suxiangjing 1 (China, [33])	A total of 0.5 mg kg^{-1} DW soil selenite applied to the soil at different growth stages of rice (i.e., seedling, tillering, booting and mature stages)	Pot experiments contained two separate soils: neutral (0.30 mg Se kg^{-1} , pH 7.41) and acidic soil (0.37 mg Se kg^{-1} , pH 5.02), the highest concentration of Se in rice was found during the booting stage, SeMet was predominate (90% organic species) in rice
III. Maize plants (<i>Zea mays</i> L.)		
Maize: <i>Zea mays</i> L. Dekalb DKC4316, FAO 300 (Italy, [142])	Applied-Se through fertigation at a rate of 200 g of Se ha^{-1} as sodium selenite under high and low water regimes	Field experiment, soil total Se content was 0.25 mg kg^{-1} soil, Se-fortified maize enhanced nutraceutical value and antioxidant content of grains
Maize: <i>Zea mays</i> L. Dekalb DKC4316, FAO 300 (Italy, [143])	Se was applied as Na_2SeO_3 through fertigation at rate of 100 g ha^{-1} twice under low and high irrigation regimes	Field experiment, total soil Se content was 0.183 mg kg^{-1} soil, soil Se did not leach into groundwater but was lost over time through volatilization process
Varieties: KH 600-15A, KH 500-33A and K132 (Kenya, [125])	Soil and foliar -Se fertilizer applied at 5, 10, and 20 g Se ha^{-1} as sodium selenate, the mean of total Se was 0.345 mg kg^{-1} for all locations	Field experiments were carried out at three locations (Mbuyu, Mbeu and Kiaga), the Se level of biofortification (0.3 mg kg^{-1}) was achieved in Kiaga and Mbeu using a foliar Se-dose of 20 g ha^{-1}
Cultivar: Agaiti-2002 (Pakistan, [146])	Foliar sprayed with sodium selenate (20 and 40 mg L^{-1}) under NaCl salt stress ($\text{EC} = 12 \text{ dS m}^{-1}$) at both reproductive and vegetative stages	Pots filled with washed river sand, plants harvested 10 days after foliar spraying, foliar Se level of 20 mg L^{-1} was more effective at inducing salt tolerance in maize plants
Cultivar: Nongda 108 (China, [147])	Added Se at 1, 5, and 25 $\mu\text{M Na}_2\text{SeO}_3$, 15 days after treatments, antioxidative capacity, and biomass determined	Pots filled with vermiculite, induced salinity stress (NaCl 100 mM), application of 5 μM Se may alleviate the adverse effects of salt stress
Variety: Luyuan 502 (China, [64])	Foliar applied 30 g Se ha^{-1} in multiple forms: sodium selenite, selenate, seleno-methionine, and chemical nano-Se	Field experiment, soil total Se 0.46 mg kg^{-1} , pH 7.82, residual effect of Se applied on wheat was studied on maize in the following year

3.2. Biofortification of Vegetable Crops: Tomato, Potato and Lettuce

Vegetables are a major source of nutrients and phytochemicals that support human health and nutritional sustainability [148]. Vegetables routinely grown for human consumption include allium, cruciferous, legumes, yellow-orange-red, and leafy green vegetables [149]. These vegetables are important in biofortification programs due to their importance for human health and short growth period (Table 3). Several vegetable crops have already been used in biofortification programs, including

vegetables enriched in Se such as tomato [150,151], potato [85,94,120], lettuce [91,126,152], onion [153], garlic [154,155], cabbage [139], carrot, broccoli [156,157], asparagus [158], radish [66,159,160], and spinach [30,158].

Table 3. Some selected vegetable crops (tomato, potato, and lettuce) that have been investigated for selenium biofortification.

Plant Cultivar (Country, Reference)	Selenium Forms and Added Rate	Experimental Conditions and Se-Biofortified Dose
I. Tomato plants (<i>Solanum lycopersicum</i> L.)		
Tomato seeds from Yangling Longkind Hondings Co., Ltd. (China, [69])	45 days after transplanting Se added as Na ₂ SeO ₃ and/or Na ₂ SeO ₄ at 0.01, 0.05, 0.1, 0.5, and 1 mg Se L ⁻¹ for 7 days exposure	Under hydroponic culture, Se concentration at 0.05 mg L ⁻¹ with both selenate and selenite as Se sources was the optimum for tomato fruit Se content
Cultivar: Red Bunch (Italy, [151])	Sodium selenate added into the nutrient solution at a rate of 1 and 1.5 mg Se L ⁻¹ , 14 days after transplanting	In greenhouse seedlings were transferred into rock wool blocks under drip irrigation, fruit quality and fruit shelf life performance during postharvest and storage were improved using 1.5 mg Se L ⁻¹
Seeds of cultivar Micro-Tom and genotype high pigment-1 (Brazil, [161])	At 3-weeks old, 50 µM Na ₂ SeO ₃ applied, the exposure of seedlings was for 74 days	Cultivated in boxes containing a mixture of pot mix and vermiculite (1:1 by volume), Se may restrict Cd uptake (0.5 mM CdCl ₂) improving fruit quality
Cultivar: Provence (China [150,162,163])	A foliar spray applied at 1 mg Se L ⁻¹ as sodium selenate 4 weeks after transplanting	In a greenhouse, sandy soil (49% sand, soil pH 6.5), harvested at pink stage from Se treated, Se (1 mg Se L ⁻¹) may delay ripening, improve fruit nutritional quality
II. Potato plants (<i>Solanum tuberosum</i> L.)		
Cultivar: E-potato 10 (China, [85])	Applied-Se through foliar spraying at 100 g·ha ⁻¹ as sodium selenate and selenite, plants harvested after 92–95 days	Field experiment, two locations, total Se: 0.26 and 0.35 mg·kg ⁻¹ soil, applied at three growth stages, selenate applied at the tuber bulking stage of potato had the highest tuber Se-content
Cultivar: Sante (Pennsylvania, USA, [164])	One week after planting, seedlings treated with 9 µM Se as sodium selenate and harvested after 55 days	Pots in growth chamber filled with fine acid-washed sand, pots treated by adding Cd (40 µM) and/or As (40 µM), Se may reduce Cd and As toxicity
Cultivar: Agata (Brazil, [94])	Selenium was applied together with planting fertilization using 0.75, 1.5, 3, and 5 mg kg ⁻¹ as sodium selenate and selenite	Pots filled with tropical soil (pH 4.8; clay 71%; total Se content 0.065 mg kg ⁻¹), selenate at low dose (0.75 mg kg ⁻¹) was the most efficient source for potato biofortification under tropical conditions
Cultivar: Vineta (Poland, [120])	Applied Se as Na ₂ SeO ₃ at 0.5 mg Se·L ⁻¹ (i.e., 6.3 µM Se), in the presence of 5 mg I L ⁻¹ , and 0.1, 1 and 10 mg L ⁻¹ salicylic acid	NFT hydroponic system, applied iodine + Se (0.5 mg Se·L ⁻¹) and also salicylic acid may increase N and K in tubers but decrease Mn and Zn content in roots
III. Lettuce plants (<i>Lactuca sativa</i> L.)		
Lettuce var. crispa cv Veneza Roxa (Brazil, [126])	Added selenate- and selenite (25 and 40 µmol L ⁻¹ Se), plants harvested after 28 days from cultivation	In hydroponic system, the Se-bioavailability was higher for selenite (40 µmol L ⁻¹ Se) compared with selenate due to its fast bio-transformation into organic forms in plant cells
Six different varieties (Poland, [91])	Seedlings fortified with Na ₂ SeO ₃ at 0.5 mg Se L ⁻¹ , with and without 5 mg I L ⁻¹	In greenhouse, seeds sown into mineral wool plugs, seedlings placed into the NFT hydroponic method, salicylic acid improved bio-fortification efficiency using Se and I
Seeds of lettuce (Rijk Zwaan, the Netherlands, [165])	Applied Se at 0.1, 0.5, 5, 10, and 50 µmol L ⁻¹ as Na ₂ SeO ₃ for 30 days	Hydroponic system, the 0.5 µmol L ⁻¹ Se may be used to reduce nitrate content in leaves with increasing lettuce yield, inducing assimilation of NO ₃ ⁻
Cultivar: Veneza Roxa (Italy, [166])	10, 25, and 40 µmol Se L ⁻¹ as sodium selenate or selenite added to solution, harvested after 28 days	Hydroponic system with pH adjusted between 5.5–6.5 daily, Se accumulation in lettuce leaves was highest in the case of selenate (bioaccessibility 70%), Selenite enriched lettuce was more favorable at lowest fortification level (10 µmol Se L ⁻¹)
Hungarian cultivar: Susana (Egypt, [152])	Soil and foliar applied Se at 50, 75, and 100 mg kg ⁻¹ in the form HNaO ₃ Se	Field experiment, salt-affected soil (pH: 8.65; EC: 4.49 dS m ⁻¹ ; clay: 53.3%; total Se content: 0.050 mg kg ⁻¹), foliar application of 100 mg kg ⁻¹ is the best treatment under this salinity stress
Variety: capitata (Poland, [91])	Selenium fortified seedlings at 0.5 mg Se L ⁻¹ as Na ₂ SeO ₃ , with and without 5 mg I L ⁻¹	NFT or dry hydroponic method, salicylic acid applied at 0.1 mg·L ⁻¹ may increase the leaf content of selenomethionine under enrichment with I and Se

Abbreviation: NFT (nutrient film technique).

For tomato crops, the Se-biofortification program may differ depending on the growth media. A Se concentration of 0.05 mg L^{-1} with selenate and selenite as dual Se sources may be optimum for tomato fruits grown under the hydroponic technique [69], but under drip irrigation this dose may be up 1.5 mg Se L^{-1} [151]. For potato crops, foliar applied Se at 100 g ha^{-1} at the tuber bulking stage led to the highest tuber Se-content [85], whereas a low Se dose (0.75 mg kg^{-1} as selenate) for pots filled with tropical soil (pH 4.8; clay 71%; total Se content 0.065 mg kg^{-1}) was the most efficient source for potato biofortification under tropical conditions [94]. The hydroponic system is a common technique for producing lettuce under greenhouse conditions and the Se-bioavailability in a hydroponic system was higher for selenite ($40 \text{ } \mu\text{mol L}^{-1}$ Se) compared with selenate due to its fast bio-transformation into organic forms in plant cells [126]. Under field conditions, a foliar of rate of 100 mg kg^{-1} may be the proper Se dose for lettuce biofortification in salt-affected soils (pH: 8.65; EC: 4.49 dS m^{-1} ; [152]).

4. Interaction of Selenium and Nano-Selenium with Environmental Conditions

Plant growth and development are mainly controlled by environmental conditions (e.g., water, nutrients, air, light, etc.). These conditions include both normal and stressful environments (i.e., biotic and abiotic stresses). Plants have the ability to alleviate these stresses using exogenous and endogenous anti-stressors or tools (through their defense system) such as plant growth-promoting rhizobacteria [167], silicon [168–170], nanoparticles of selenium, or silicon [171] and selenium [2]. Several studies have confirmed the identified role of Se in promoting cultivated plant growth under a variety of stresses [24,83,86,172,173]. There is a growing body of literature that shows the potential of Se-nanoparticles to promote plant growth under different conditions (Table 4), but this still needs more investigation, particularly under stressful conditions. Comparing the potential of Se-NPs as revealed in human and animal studies with plant investigations, much more progress has been achieved in the human and animal fields.

It is well documented that biological nano-Se has desirable characteristics such as high biosafety and bioactivity properties, low toxicity, high solubility, and high mobility due to their large surface/volume ratio compared with soluble inorganic Se salts, mainly selenate and selenite [24,34]. The uptake of Se-NPs by plants primarily depends on the synthesis method (bio-synthesis or chemo-synthesis) and the size of the nanoparticles [141].

Table 4. The role of selenium-nanoparticles (Se-NPs) in the growth of selected plants under different growth conditions.

Plant Cultivar (Country, Reference)	Nano-Se and Added Rate	Experimental Conditions and Se-Biofortified Dose
Groundnut: <i>Arachis hypogaea</i> L. three cultivars: NC, Gregory and Giza 6 (Egypt, [35])	Foliar applied Se-NPs (10–30 nm) at 20 and 40 mg kg^{-1} during vegetative stage, plants harvested 45 days after planting	Pot experiment with sandy soil (pH 8.2), Se-NPs may act as stimulator and/or stressor, enhanced the antioxidant defense systems under sandy soil conditions at 20 mg kg^{-1}
Tomato (<i>Solanum lycopersicum</i> L.), type saladette El Cid F1 from Harris Moran, Davis, CA, USA (Mexico, [58])	Foliar applied Se-NPs (2–20 nm) at 1, 10, and 20 mg L^{-1} in combination with Cu-NPs (40 nm) at 10, 50 and 250 mg L^{-1} , harvested fruits at light red stage 102 days after transplanting	Under multi-tunnel greenhouse, bags filled with potting mix (1:1 (v/v) peat moss and perlite), Se-NPs (10 mg L^{-1}) alone recorded the highest yield (increase up to 21%). Combination with Cu-NPs (50 mg L^{-1}) improved antioxidant system and fruit quality (vitamin C, flavonoids, firmness and glutathione)
Tomato (<i>Solanum lycopersicum</i> L.), type saladette El Cid F1 from Harris Moran, Davis, CA, USA (Mexico, [59])	Foliar applied Se-NPs (2–20 nm) at 10 and 20 mg L^{-1} and Cu-NPs at 10 and 50 mg L^{-1} , harvested fruits at light red stage	In multi-tunnel greenhouse, bags filled with potting mix (1:1 (v/v) peat moss and perlite), Se-NPs (20 mg L^{-1}) may enhance non-enzymatic antioxidants under stress from the fungal pathogen (<i>Alternaria solani</i>), Cu-NPs may also enhance this effect
Tomato (<i>Solanum lycopersicum</i> L.), type saladette El Cid F1 (Mexico, [60])	Se-NPs (2–20 nm) added at 1, 5, 10, and 20 mg L^{-1} every two weeks, harvested fruits at light red stage	In multi-tunnel greenhouse, polyethylene bags filled with potting mix (1:1 (v/v) peat moss and perlite), Se-NPs (20 mg L^{-1}) increased antioxidant compounds (lycopene, flavonoids, β -carotene and phenols) in fruits and fruit quality under salt stress (50 mM NaCl)
Pomegranate: <i>Punica granatum</i> , cv. Malase Saveh, trees 10 years old (Iran, [23])	Foliar applied sodium selenite and Se-NPs (10–45 nm) on the upper surface of leaves at 1 or $2 \text{ } \mu\text{M}$ for both Se forms	In field experiment, soil (sand 58%, pH 7.8), trees were sprayed twice one week before the first full bloom, Se ($1 \text{ } \mu\text{M}$) and Se-NPs ($2 \text{ } \mu\text{M}$) promoted maturity index and quality of fruits

Table 4. Cont.

Plant Cultivar (Country, Reference)	Nano-Se and Added Rate	Experimental Conditions and Se-Biofortified Dose
Strawberry (<i>Fragaria ananassa</i> Duch.), cv. Kurdistan (Iran, [24])	Foliar applied Se-NPs (10–45 nm) at 10 and 20 mg L ⁻¹ , the plants were harvested about 80 days after planting	In pots filled with perlite, coco peat and sand (5:7:23, w:w:w), Se-NPs (20 mg L ⁻¹) mitigated soil salinity stress (up 75 mM NaCl), and improved plant salinity tolerance
Wheat: <i>Triticum aestivum</i> L., var. Pishtaz (Iran, [87])	Germinated seeds via foliar applied Se-NPs (10–45 nm) at 5, 10, and 50 mg L ⁻¹ for 7 days then harvested	10-day-old seedlings in pots containing peat and perlite (1:1), Se-NPs (5 mg L ⁻¹) enhanced nitrate reductase activity, and peroxidase
Sorghum (<i>Sorghum bicolor</i> L. Moench), genotype DK 28-E (India, [34])	Se-NPs (10–40 nm) at 10 mg L ⁻¹ added to seedlings	Washed sand soil, foliar applied Se-NPs during the booting stage of sorghum under heat stress, Se-NPs (10 mg L ⁻¹) stimulated antioxidant defense system via enhancing activity of antioxidant enzymes
Wheat (<i>Triticum aestivum</i> L.), cv. Luyuan 502 (China, [141])	Used 5 µM chemical Se-NPs (40, 140, 240 nm) and biological Se-NPs (using <i>Rahnella aquatilis</i> HX2), compared with selenite	Seedlings were cultivated in greenhouse in plastic containers for 6 weeks, treatments were exposed to Se for 72 h, Se-NPs could be applied as a new fertilizer to produce Se-biofortified plants with best root uptake for 40 nm Se-NPs
Spinach: <i>Spinacia oleracea</i> L., Stoik variety (Russia, [30])	Se was foliar applied in three forms (220 g Se ha ⁻¹) including Se-NPs (<10 nm), selenite and selenate, harvested after 60 days	In field experiment in heavy loamy soil, pH 6.8, Se-NPs can be used in the biofortification of spinach with Se due to higher growth-promotion compared to other Se forms
Tobacco: <i>Nicotinia tabacum</i> L. cv. Ottawa, Petit, Havana (Hungary, [174])	Sodium-selenate or red nano-Se added at 0.53; 5.3; 53; 265; 530 µM into MS medium	Seeds were sown onto hormone-free MS medium, tobacco callus cultures can uptake Se-NPs (265–530 µM) stimulating growth of the root system

5. Selenium and Nano-Selenium Reduce the Toxicity of As, Cd, and Hg

Selenium has long been of great interest in a wide range of fields including the medical, pharmaceutical, agricultural, and industrial sectors. Recently, a considerable literature has developed around the potential of Se and Se-NPs to help humans and animals deal with environmental stresses, but much less research has been done regarding Se, Se-NPs, and environmental stress in plants [175]. Plant related soil and environmental stresses include salinity, drought, waterlogging, heat, and heavy metal stress, which represent serious constraints for global crop productivity [109]. Heavy metals and potential toxic trace metals that may exist in soil include arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), and selenium, which can be introduced into the soil through human activities such as mining [176], industrialization [177], urbanization [33], and agricultural practices [178,179]. These metals can also naturally occur in soils at levels that may cause problems [28,105].

Several investigations suggest that Se can play an important role for plants growing under stressful conditions, although further work on Se is required to confirm its essentiality for higher plants. Many studies conducted on Se and its potential in higher plants have investigated its uptake, translocation, metabolism, and toxicity [17,160,180,181]. Biofortification studies with Se and Se-NPs are considered promising because Se is essential for human and animal health [53]. Selenium biofortification and phytoremediation are at the same level of importance from the environmental protection point of view [81]. Based on the work done to date, some general comments can be made concerning the role of Se and nano-Se in higher plants under different stresses:

1. The ameliorative role of Se when plants are stressed by soil heavy metal content has been investigated in several studies, including how Se protects plants against heavy metal stress, whereas there is little work investigating the use of Se-NPs in this context [34,78].
2. Selenium has already been investigated by many researchers as a way to ameliorate As stress on rice plants [2,92,182–191]. These studies primed rice seed with Se during germination under As-stress. The ameliorative role of Se under As-oxidative stress occurred through the modulation of thiol (R-SH) and antioxidant enzymes in rice or Se-modulating the level of phenolics and nutrients alleviating the toxicity of arsenic in the rice plants.
3. The most important studies investigating Se and its role under Cd stress on rice plants include the application of Se to mitigate Cd accumulation in high-Cd-contaminated soils [192], the behavior of Se at different planting times [115], the effects of Se-forms and application methods on modulation

- of rice growth [83], how Se reduces the uptake and translocation of Cd from contaminated soils [193], and reducing oxidative stress induced by Cd [33,194–198].
4. Selenium has been shown to moderate the impacts of mercury (Hg) stress on cultivated rice [78,138,199–201]. Chapman et al. [202] studied how native plants in a mined field were able to grow under Hg and As soil pollution as well as how selenium promoted the growth of the plant seedlings by decreasing Hg and As bioaccumulation in these plants. Selenium also has the ability to decrease rice plant uptake of methylmercury in Hg-contaminated soils while increasing the uptake of other nutrient elements [78].
 5. Selenium can alleviate chromium (Cr) stress in many crops by regulating the Cr uptake. Research into this relationship has included Chinese cabbage [203], pak choi (*Brassica campestris* L. ssp. *Chinensis* Makino) [204], and mitigating Cr-toxicity in *Brassica napus* L. [205], *Brassica juncea* seedlings [206], and cabbage (*Brassica campestris* L. ssp. *Pekinensis*) [207].
 6. Studies have demonstrated the mitigation of lead (Pb) toxicity by Se-application in ginger (*Zingiber officinale* Roscoe.) [208] and oilseed rape (*Brassica napus* L.) plants [209].
 7. Selenium nanoparticles are also thought to behave like Se in protecting cultivated plants under heavy metals stress but only a few studies have been published. Investigations that have been conducted regarding Se-NPs and stressful environments include the role of Se-NPs in enhancing the growth of some cultivated plants under stress such as sorghum under high temperature stress [34], strawberry under salinity stress [24] and rice under Cd and Pb toxicity [171].

6. Are Selenium and Nano-Selenium Emerging Pollutants?

With widespread interest in Se biofortification, it is possible that the environment (i.e., soil, water, air, and plants) might become contaminated with Se due to extensive Se applications, which in turn could create a risk for human health [28,210] (Figure 2). The main anthropogenic sources of Se-pollution include agricultural and industrial activities [211–213]. The most serious problem resulting from Se-pollution is contamination of drinking water sources; hence, remediation requires effective techniques to remove Se from natural waters [214,215]. Environmental Se-contamination may be bio-remediated using proper microbial adaptations like alkylation [216] or Se-transformation, bioavailability, mobility and volatilization into the atmosphere [15].

In addition to anthropogenic Se contamination problems, soils that are naturally high in Se content (seleniferous soils) are found in several places worldwide such as Punjab, India [217–219], Pine Ridge, Fort Collins, Colorado, USA [220], Western Colorado, USA [221], Enshi in China [222,223], Saskatchewan in Canada, Queensland in Australia, Irapuato in Mexico, the State of Boyaca in Colombia, and the Deog-Pyoung area in Korea [224]. Therefore, to address both anthropogenic and natural Se problems in soil, there is an urgent need to study Se-biofortification and phytoremediation through hyper-accumulating plants, which have the ability to uptake huge amounts of Se from soils and transfer it into the atmosphere by volatilization [67]. The agronomic Se biofortification of edible plants, pasture and forage crops should be improved through judicious biofortification programs without excessive applications or applications of Se in places that do not experience Se deficiency in the soil. Over-biofortification of products with selenium should be avoided, even in areas with Se-deficiencies, to prevent the build-up of Se to toxic levels in food and related products.

Further investigations are needed to monitor the behavior of Se-NPs in different environments including agricultural soils, waters, and farm animals. Se-NPs have already been used in the remediation of soils and waters that were contaminated with heavy metals like mercury [225–227]. This work confirmed that soils and groundwater contaminated with elemental mercury could be remediated via biogenic Se-NPs based on mechanisms like the immobilization of elemental mercury [226,227], applied hetero-aggregation of soil particulate organic matter [225], applied biofilm-coated quartz sand [228], and applied goethite colloids [138] in the presence of Se-NPs [64].

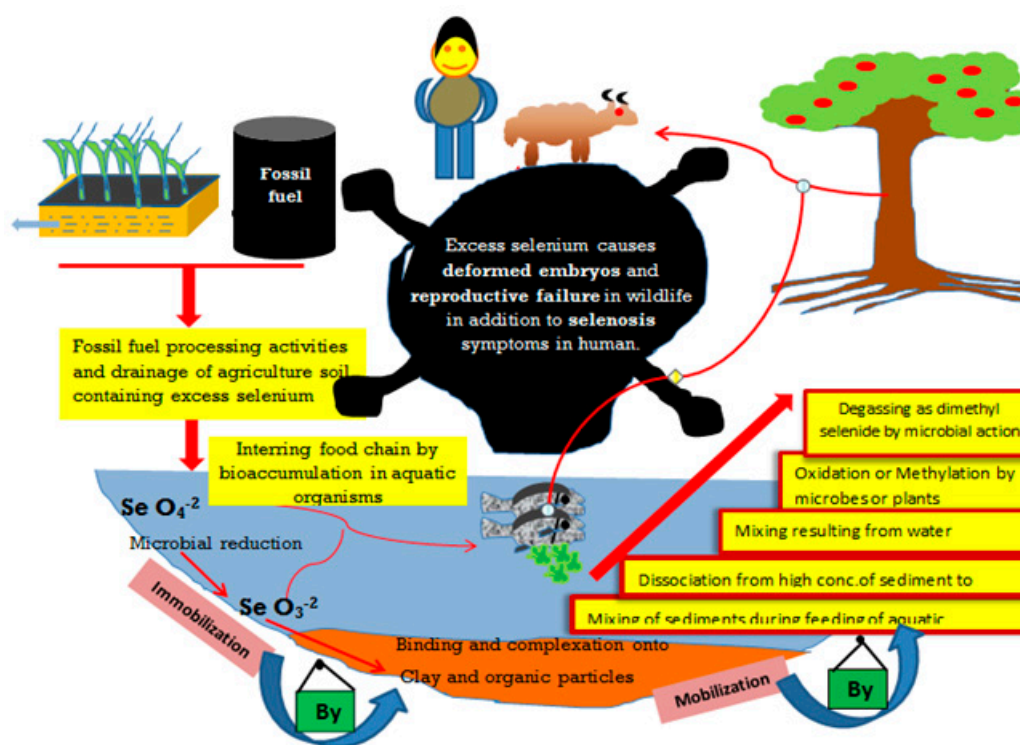


Figure 2. Possible fates of selenium in the environment. Selenium could be released into the aquatic environment from industrial and agricultural activities. The excessive application of Se as fertilizer may lead to environmental hazards, which may be controlled by immobilization and mobilization reactions in soil, driven by soil clay and organic matter content as well as microbial activities. These Se-pathways may threaten human and animal health.

7. Conclusions

Selenium is an essential micronutrient for the nutrition of humans, animals, and lower plants, but whether or not it is essential for higher plants has yet to be confirmed. Nano-selenium is one of the most potentially useful Se-forms and has fascinating properties that lead to its use in nanomedicine applications, drug delivery, therapeutic applications, biomedical applications, and cancer prevention. Several places worldwide have a Se-deficiency problem due to low Se soils and should implement programs that guarantee safe and proper Se-supplementation levels for human health. The biofortification approach has shown particular promise for dietary supplementation of Se so that humans and/or animals have Se present in the right form, place, dose, and time. Due to the changing world and environmental stressors, there is an urgent need to document how and when Se helps plants and animals overcome stresses, including the identification of suitable Se and Se-NP fertilizers, application timing, and application rates. Several studies have demonstrated the ameliorative effects of Se and nano-Se on plants and farm animals under stress, but the use of Se-NPs in biofortification programs still needs more research. Despite all the documented benefits of Se, the difference between Se deficiency and Se toxicity is very small. We also need to understand the possible adverse effects and cytotoxicity of these Se-NPs on humans and effects on crops. It is also important to understand Se and Se-NP accumulation, transformation, and transport through soil. Due to the intensive use of Se and its forms in many fields, Se and nano-Se have become potential emerging pollutants in agroecosystems. This issue has increasingly been recognized as a potentially serious, worldwide public health concern.

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