



Invited review

Review of Pb availability and toxicity to plants in relation with metal speciation; role of synthetic and natural organic ligands

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ABSTRACT

Biogeochemical behavior of lead (Pb), a persistent hazardous pollutant of environmental concern, strongly depends on its chemical speciation. Therefore, in this review, link between Pb speciation: presence of organic ligands and its environmental behavior has been developed. Both, biogeochemical and ecotoxicological data are discussed in environmental risk assessment context and phytoremediation studies. Three kinds of organic ligands selected for this review include: (1) ethylene diamine tetra-acetic acid (EDTA), (2) low molecular weight organic acids (LMWOAs) and (3) humic substances (HSs). The review highlights the effect of Pb speciation on: (i) Pb fate and behavior in soil; (ii) Pb plant uptake and accumulation in different plant parts; and (iii) Pb-induced phyto-toxicity. Effects of organic ligands on Pb speciation are compared: how they can change Pb speciation modifying accordingly its fate and biogeochemistry in soil-plant system? EDTA forms soluble, stable and phytoavailable Pb-chelates due to high binding Pb affinity. LMWOAs can solubilize Pb in soil by decreasing soil pH or increasing soil organic contents, but have little effect on its translocation. Due to heterogeneous structure, HSs role is complex. In consequence Pb speciation knowledge is needed to discuss phyto-toxicity data and improved soil phytoremediation techniques.

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Contents

1. Introduction	2
2. EDTA	2
2.1. Effect of EDTA on Pb speciation and behavior in soil	3
2.2. Effect of EDTA on Pb plant uptake and accumulation in different plant parts	3
2.3. Effect of EDTA on Pb-induced toxicity to plants	4
3. LMWOAs	5
3.1. Effect of LMWOAs on Pb speciation and behavior in soil	5
3.2. Effect of LMWOAs on Pb plant uptake and accumulation in different parts	5
3.3. Effect of LMWOAs on Pb-induced toxicity to plants	6
4. HSs	6
4.1. Effect of HSs on Pb speciation and behavior in soil	6
4.2. Effect of HSs on Pb plant uptake and accumulation in different plant parts	7
4.3. Effect of HSs on Pb-induced phyto-toxicity	7
4.4. Use of speciation models to calculate probable application value of organic ligands	8
5. Conclusions and perspectives	8
Acknowledgments	9
References	9

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1. Introduction

Despite considerable progress in recent years, Pb speciation in ecosystems, its transfer to the biosphere as well as associated ecological risks are still topical and remain an important research area [1,2]. Several previous studies and reviews have reported that Pb is hazardous material of environmental concern with high persistency. This metal has no biological function and can induce, directly or indirectly, various morphological, physiological and biochemical dysfunctions in plants such as decrease in seed germination, plant growth, chlorophyll production. Pb also causes lipid peroxidation, oxidative stress and DNA damage in plants [2–11].

However, biogeochemical Pb behavior in ecosystems and its potential effects on plants are strongly influenced by its speciation [2,12–15]. Speciation is the relative existence of a metal in different chemical forms under different environmental conditions. Pb in soils exists in several forms with different levels of solubility and bioavailability as follows: (i) dissolved (in soil solution), (ii) exchangeable (in organic and inorganic components), (iii) structural components of the lattices in soils and (iv) insolubly precipitated with other soil components [16–19]. Usually, only the first two forms are considered as phytoavailable. In contrast, Pb which is an intrinsic part of the solid-phase minerals may not be phytoavailable [16]. This implies that Pb solubility and mobility in soil and plant uptake depends on its chemical speciation [12]. Several previous studies have described that free Pb cations concentration correlates better with its uptake and toxicity than total Pb concentration [20,21]. This makes total concentration, although easier to measure, an unreliable indicator of toxicity [2,22]. Therefore, predicting the relevant species of Pb is very important step in improving our understanding of risk assessment or remediation studies.

Interactions between organic ligands and metals in natural media have been particularly studied due to their high affinity for metals [23–25]. Synthetic or natural organic ligands such as diethylene triaminepenta-acetic acid (DTPA), glycoetherdiamine tetra-acetic acid (EDGA), ethylene diamine tetra-acetic acid (EDTA), ethylene diamine disuccinate (EDDS), nitrilo tri-acetic acid (NTA), low molecular weight organic acids (LMWOAs) and humic substances (HSs) have been used extensively in Pb remediation techniques and to enhance micronutrient supply to plants [5,26–28]. These organic ligands desorb Pb from soil matrix into soil solution and facilitate its uptake by plant roots and translocation to shoot tissues at varying degree. Indeed, these ligands restrict Pb ions from playing their

normal chemical roles by forming complexes [2]. Fu and Wang [29] reviewed the interaction between different chemical processes and organic ligands controlling Pb and other heavy metal kinetic in soil solution. The organo-metallic complexes vary greatly in term of stability, solubility and chronic toxicity compare to free metal ions [2,30]. Many previous studies have presented the changes (increase or decrease) induced by organic ligands in Pb solubilization, uptake, translocation and acute toxicity associated with Pb [2,27,30–33]. Fig. 1 compares the effect of three types of organic ligands (EDTA, LMWOAs and HSs) on Pb solubilization in soil and uptake by plant.

Therefore, in order to better understand environmental biogeochemical Pb behavior it's necessary to make a link between its chemical speciation, mobility, solubility, phytoavailability and phyto-toxicity. How Pb chelation by organic ligands changes its speciation and in turn its biogeochemical environmental behavior is described in this review. Three types of organic ligands widely used in literature were selected on the basis of contrasted influence and nature: EDTA as model of synthetic chelating agents, LMWOAs as model of root exudates and HSs as natural dissolved organic matter (DOM) present in soils.

2. EDTA

EDTA was patented in Germany in 1935 by Munz [34] and was first reported by Wallace et al. [35] for its ability to increase water-soluble metal concentrations in soils. EDTA is a hexahydric acid, which forms Pb-chelates. Strong Pb-EDTA interactions originate from six strong donor atoms in EDTA (two nitrogen atoms of amines and four oxygen atoms of carboxylates). These donor atoms surround Pb cation in the complex in order to achieve the maximal number (6) of possible donor–acceptor interactions [36]. In addition, EDTA anionic character also contributes to complex stabilization. Particularly, the four carboxylate oxygens can establish strong electrostatic attraction with the captured Pb cation. Pb-EDTA complex structure obtains octahedral shape in which two oxygens occupy axial positions, while the other two oxygens and the two nitrogens form the equatorial section while the Pb at the center [36].

Many authors reported EDTA as the most efficient in increasing Pb solubility and phytoavailability in soils [2,5,37]. It has been therefore widely used in remediation of Pb and other toxic metal polluted soils [38–41]. EDTA is also applied in chelation therapy for solubilization of metals, as well as in numerous industrial

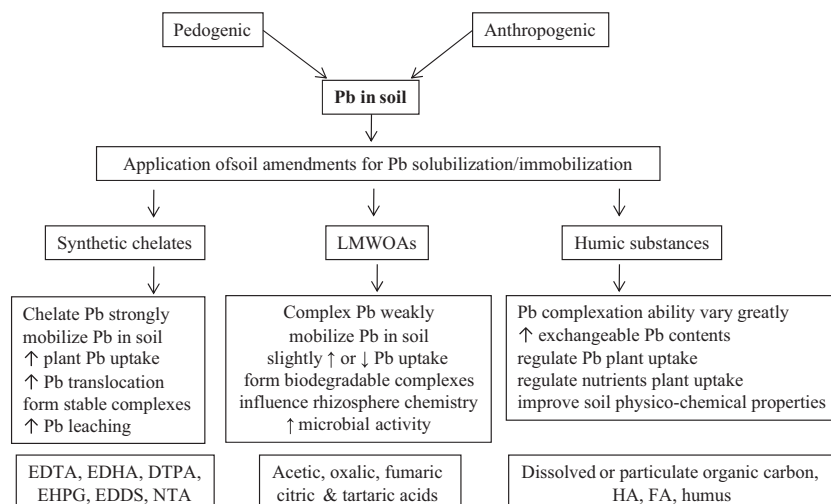


Fig. 1. Comparison of different organic ligands towards their role in Pb solubilization in soil and uptake by plants.

applications such as washing and cleaning agents [36,42]. Additional important applications of EDTA complexes appear as diagnostic and therapeutic agents for treating metabolic disorders and diseases [36]. Due to these numerous applications, EDTA is currently detected in the environment.

2.1. Effect of EDTA on Pb speciation and behavior in soil

Metal bioavailability is defined as the fraction of total metal soil content that can be absorbed by a biological target. Despite high total Pb soil concentration, only a low Pb fraction is readily available for plant uptake in the absence of organic ligands [3,15]. This is because of most portions of the Pb in soil occur in residual form [39,43–46] due to strong complexation with no biotic or biotic ligands [14,15,18,19]. Therefore, Pb in soil is thought to be low soluble and plant available due to its immobile nature [14,47–49].

In general, mobility and availability of Pb in soils are controlled by adsorption and desorption phenomena [50–56], which in turn are associated with physico-chemical soil properties [57,58]. Presence of EDTA increased Pb exchangeable fraction in soil. Pb solubilization results of a competition between adsorption by solid surfaces and formation of EDTA complex. According to many reports, EDTA can release Pb from several soil compartments, particularly Pb associated to Fe and Mn oxides and to organic matters [59–61]. Several authors observed a linear relationship between EDTA concentration and Pb mobilization in soils [43,59]. When EDTA is applied to soil, formation of Pb–EDTA complexes shift precipitation and sorption equilibrium toward increased dissolution of Pb [39,43,62] due to high binding affinity of EDTA for Pb ($pK = 17.8$). Wu et al. [63] and Nascimento et al. [64] respectively noted a 600- and 217-fold increased Pb concentration in soil solution by EDTA.

However, EDTA effect on Pb solubilization in soils greatly varied according to following factors: soil physico-chemical properties (texture/structure, pH, redox potential, cation exchange capacity, soil buffering capacity, fixation/aging) [15,33,41,59,65,66], soil biological and microbial conditions [15,67–69], concentration of other competing metals and EDTA [33,42,70], Pb:EDTA stoichiometric ratio [15,33,66,], soil exposure time to contaminants, time and mode of EDTA application [2,63,67] and combined application of EDTA with other amendments [15,71–74]. These numerous influencing parameters could explain why it can appear contrasted and sometimes opposite results when comparing several studies dealing with EDTA–Pb–soil systems.

It has been reported that the presence of competing cations Ca^{2+} , Mg^{2+} , Fe^{2+} , Al^{3+} , Zn^{2+} , Cu^{2+} , Cd^{2+} considerably modifies Pb complexation by EDTA due to the competition for binding sites of EDTA [75]. The quantity of Pb extracted/complexed by EDTA also varies with the type of soil matrix [28,76]. The complexation of Pb by EDTA may reduce to half in calcareous soils compared to non-calcareous soils [75]. Soil pH also affects the efficiency of EDTA towards metal solubilization in soil [77]. EDTA is most effective under acidic and neutral conditions whereas its metal binding efficiency decreases with increase in pH [102]. The combined application of EDTA with pH lowering agent such as citric acid or elemental sulfur is reported to increase Pb solubilization in soil [78]. All these soil and metal factors separately or in combination with each other affect metal complexation by EDTA. An understanding of these factors and mechanisms is therefore important in assessment and management of risks associated with Pb contaminated soils. By knowing these factors, it is possible to predict the retention and adsorption level of Pb and other metals in soil. Soil physico-chemical characteristics also help to determine the absolute threshold levels of Pb and other heavy metals in soils above which plant metal uptake will become a concern to plant and human health.

2.2. Effect of EDTA on Pb plant uptake and accumulation in different plant parts

Although, Pb forms stable complexes and tend to store, mainly, in soil but a mobile fraction can be absorbed by living organisms [18,19] and go well in food chain. EDTA is known to increase Pb uptake and translocation from roots to aerial parts, which is one of the most prominent features of its utilization in phytoremediation. Increased Pb uptake by EDTA has been documented in several recent studies [2,15,28,39,41]. This increase in Pb uptake by plant roots is due to formation of Pb–EDTA complex, which is readily taken up by most of plant species [2,79]. It depends on substances released by roots including organic acids, types and varieties of plants, plant maturity [57,80] and planting density [81]. Different authors reported a wide variation in EDTA-induced increased Pb uptake by plants, i.e., Andra et al. [38] reported 15 times increased Pb uptake by *Vetiveria zizanioides* roots, whereas Sun et al. [39] demonstrated 161-fold increased Pb accumulation by *Juncus effusus* roots. Table 1 depicts effect of EDTA addition on Pb uptake and translocation by different plant species.

Several studies have shown that without chelators, major part of Pb (almost 95%) tends to accumulate in root cells. This Pb translocation restriction phenomenon is due to precipitation as insoluble Pb-salts or immobilization by the negatively charged pectins within cell wall, precipitation in intercellular space, blockage by Casparian strip, accumulation in plasma membrane or sequestration in the vacuoles of rhizodermal and cortical cells [69,82–85]. Lead accumulation in roots has been very well documented in many plants such as *Vicia faba*, *Pisum sativum* and *Phaseolus vulgaris* [2,84,86,87], *Vigna unguiculata* [83], *Nicotiana tabacum* [88], *P. sativum*, *Lathyrus sativus* [89], *Zea mays* [4], *Avicennia marina* [90], *Sesuvium portulacastrum* and *Brassica juncea* [91], non-accumulating *Sedum alfredii* [92] and *Allium sativum* [85]. However, chelation of Pb by EDTA has been described to reduce Pb sequestration in roots by a number of workers in recent years [32,38–41,93]. López et al. [71] found that EDTA addition at equimolar concentration to hydroponic medium increased Pb translocation to leaves by about 300%. Liu et al. [79] observed that EDTA addition at 0.1 mM concentration made it possible to raise Pb translocation to shoots from 17 to 31%. Recently, Barrutia et al. [41] reported that Pb chelation by EDTA resulted in 27 times higher Pb accumulation in shoots of *Rumex acetosa*.

Several previous studies have revealed the mechanism of EDTA-induced increased translocation to shoot tissues [80]. These studies suggested that EDTA chelates Pb in nutrient/soil solution [2,40,58,94] and this chelate passes through free space of roots, which is continuous with surrounding soil solution [2]. Presence of EDTA increases the root Pb flux through the apoplast and then increase Pb shoot/root ratio [95]. This is basically due to a reduced binding of Pb to extracellular cation exchange sites of the apoplast and cell walls [96] due to neutral Pb–EDTA complex. Moreover, disruption of Casparian strip by EDTA might also be responsible for higher Pb accumulation in aerial parts [97]. Huang et al. [98] also proposed that increased Pb uptake and translocation is probably due to damaged roots by EDTA.

However, some authors also reported no significant EDTA effect on Pb uptake [99,100] and de la Rosa et al. [101] concluded that EDTA application to Pb-contaminated agar media dramatically reduced total Pb acquisition in Tumbleweed plants. This inefficiency of EDTA on Pb uptake or translocation is most probably due to absence/ineffectiveness of transporter proteins responsible for Pb translocation to shoot tissues. Recently, Shahid [102] also demonstrated that EDTA enhanced Pb uptake by *V. faba* roots but decreased its translocation to aerial tissues after 24 h of exposure time. The author reported that this reduced translocation might be due to plant nature (*V. faba* is a Pb sensitive plant), short duration period or absence of transporter proteins in the presence of EDTA.

Table 1
Effect of EDTA on Pb uptake, translocation and toxicity to plants.

EDTA	Pb	Duration	Crops	Uptake	Trans.	Effects	Culture	Ref.
2.5 mM	800 mg/kg	7 days	<i>P. sativum</i>	NM	↑ 104	↓ Shoot dry matter, necrotic leaves	Soil	[206]
5 mM					↑ 139			
2.5 mM			<i>F. esculentum</i>		↑ 65	↓ Shoot dry matter, necrotic leaves		
5 mM					↑ 71	↓ Shoot dry matter, plant deth		
2.5 mM			<i>T. aestivum</i>		↑ 22	↓ Shoot dry matter		
5 mM					↑ 31			
2.5 mM			<i>V. radiat</i>		↑ 25	↓ Shoot dry matter, high necrotic leaves		
5 mM					↑ 24			
2.5 mM			<i>B. juncea</i>		↑ 16	↓ Shoot dry matter, necrotic leaves		
5 mM					↑ 27			
2.5 mM			<i>S. bicolor</i>		↑ 13	↓ Shoot dry matter, less necrotic leaves		
5 mM					↑ 24			
2.5 mM			<i>B. rapa</i>		↑ 11	↓ Shoot dry matter, necrotic leaves		
5 mM					↑ 18			
2.5 mM			<i>Z. mays</i>		↑ 8	↓ Shoot dry matter, less necrotic leaves		
5 mM					↑ 15			
2.5 mM	57.93 mg/kg	14 days	<i>H. annuus</i>	↑ 161	↑ 7	↓ Shoot dry matter, necrotic leaves	Soil	[39]
5 mM					↑ 7			
2.5 mM			<i>H. vulgare</i>		↑ 8	↓ Shoot dry matter		
5 mM					↑ 6			
8 mM			<i>J. effusus</i>		↑ 78	↓ Plant growth and root dry biomass		
5 mM			<i>Z. mays</i>		↑ 28	↓ Shoot and root dry weight		
					↑ 26	↓ Shoot and root dry weight		
			<i>P. vulgaris</i>		↑ 50	↓ Shoot and root dry weight		
					↑ 69	↓ Shoot and root dry weight		
500 μM			<i>R. acetosa</i>		↑ 27	↑ Stomatal conductance		
15 μM			<i>V. zizanioides</i>		↑ 15	↓ CAT activity and PC synthesis		
3 mM					NM	↓ Dry matter yield		
6 mM					↑ 4	↓ Dry matter yield		
9 mM					↑ 5	↓ Dry matter yield		
3 mM					↑ 14	↓ Dry matter yield		
	108 mg/kg	28 days	<i>C. sativa</i>	NM	↑ 7	No effect on plant biomass	Soil	[31]
			<i>H. annuus</i>		↑ 13			
			<i>B. rapa</i>		↑ 4			
			<i>Z. mays</i>		↑ 9			
3 mM			<i>Z. mays</i>		↑ 1.3	↓ Root and shoot dry matter		
			<i>H. annuus</i>		↑ 1.6	↓ Root and shoot dry matter		
2 mM			<i>T. aestivum</i>		↑ 5	↑ Photosynthetic rate		
4 mM					↑ 5			
8 mM					↑ 9			
1 mM			<i>P. tomentosa</i>		↑ 2	↑ total and root/shoot dry weight		
5 mM					↑ 2.5			
10 mM					↑ 3			
1.25 mM			<i>S. drummondii</i>		↑ 12	↑ Root, shoot length, chlorophyll a		
2.5 mM					↑ 12	↓ Root, shoot length, chlorophyll a		
5 mM					↑ 27	↑ Root, shoot length, chlorophyll a		
10 mM					↑ 42	↓ Root, shoot length, chlorophyll a		
250 μM	500 mg/kg	2 days	<i>C. cardunculus</i>	NM	↑ 4	↑ Plant water transpiration	Hydroponic	[95]
500 μM					↓ 1.1	↑ Plant water transpiration		
750 μM					↓ 2	↑ Plant water transpiration		
500 μM			<i>P. radiata</i>		↓ 17	No effect on cell morphology		
100 mg l ⁻¹			<i>S. drummondii</i>		↓ 15%	NM		
1.2 mM			<i>Z. elegans</i>		↑ 1.1	↓ Root length, ↑shoot length		
2.4 mM					↑ 1.5	↑ Root length, ↑shoot length		
4.8 mM					↑ 1.2	↓ Root length, ↓shoot length		
2.4 mM					↑ 1.1	↓ Root length, ↓ shoot length		
4.8 mM					↑ 1.3	No effect on Root length, ↑shoot length		
9.6 mM					↓ 1.1	↓ Root length, ↑shoot length		

NM, not mentioned; Trans, translocation; ↑ indicates increase and ↓ indicates decrease.

2.3. Effect of EDTA on Pb-induced toxicity to plants

Excessive Pb accumulation in plant tissue is toxic to most plants [15,103]. As seen above, presence of EDTA can increase Pb plant uptake and translocation. The question asked, is therefore, what is EDTA influence on Pb-induced toxicity to plants? Liu et al. [104] reported that Pb-induced decrease in pigment contents was reduced by EDTA. Several authors concluded that Pb is phytotoxic when present in free ionic form [2,38,41,93], whereas chelated Pb forms are less phytotoxic [2,105,106]. Hernández-Allica et al. [95] reported that proper management of EDTA concentration can reduce its phytotoxicity. Table 1 presents EDTA effect on the Pb-induced phytotoxicity to various plants. Pb-induced oxidative

stress due to over production of reactive oxygen species and increase in the activities of enzymes are also reported to be prohibited in the presence of EDTA. Huang et al. [107] reported decreased Pb-induced lipid peroxidation and induction of superoxide dismutases and lysyl oxidase in presence of EDTA. Liu et al. [108] presented that EDTA inhibited Pb induction of H₂O₂, malondialdehyde, ascorbate peroxidase, superoxide dismutases and dehydroascorbate reductase, in no accumulating *S. alfredii*. Ruley et al. [106] reported that chelators mitigate Pb-induced oxidative stress by modulating anti oxidative enzyme activities in *Sesbania drummondii* seedlings. Despite higher Pb concentrations in plant tissues, amount of total thiols and catalase activity in EDTA treated vetiver tissues was comparable to control [38], showing

the protective role of EDTA against Pb toxicity. Protective role of EDTA against Pb-induced genotoxicity was also demonstrated by Shahid et al. [2] for *V. faba* root tips.

However, few authors also reported increased Pb toxicity in presence of EDTA [39,74]. Geebelen et al. [109] found reduced roots biomass, shortening of the stems and decrease in the fresh weight of *P. vulgaris* leaves when treated with Pb–EDTA complexes (10–200 mM) in Hoagland medium. Usman and Mohamed [57] reported that applying EDTA to Pb polluted soil depressed the plant growth, and significantly decreased dry matter of roots and shoots of *Helianthus annuus* plants. These authors suggested that EDTA increased Pb uptake and translocation to aerial parts and, thereby, the associated toxicity [32,40,94,110]. According to Sarret et al. [111], Pb–EDTA complex is not toxic but can dissociate after taken up by plants giving rise to increased free ionic Pb^{2+} and hence the toxicity increased. A depressed photosynthetic activity was reported when *S. drummondii* seedlings were grown in the solution culture containing 450 mg l^{-1} EDTA alone [106]. Another hypothesis to explain increased Pb toxicity in presence of EDTA could be therefore due to EDTA influence. Moreover, EDTA application may increase the potential off-site Pb migration and leaching risk into aquifers [25,61,70,112–114]. Zhao et al. [115] introduced a horizontal permeable barrier to reduce Pb and other heavy metals leaching to groundwater following EDTA application. Some authors reported that split application of EDTA [116] or in combination with other organic ligands like EDDS or NTA can minimize Pb leaching [115–119]. Moreover, EDTA and Pb–EDTA may persist for several months in field [62,117]. Recently, Wang et al. [120] proposed EDTA degradation by ozonolysis. Use of EDTA is therefore questionable and natural organic ligands could replace it for environmental uses.

3. LMWOAs

LMWOAs, which include acetic, oxalic, fumaric, citric, and tartaric acids originates from root exudation, decomposition of soil organic matter and by microbial metabolites [121–125]. These LMWOAs, can serve in soil for nutrient acquisition [126], alleviation of anaerobic stress in roots, mineral weathering and microbial attraction [127]. Recently, LMWOAs use as an alternative to synthetic chelating agents was found to be useful for Pb remediation [122,128]. These compounds induce modification in the biogeochemical Pb behavior and fate in soil directly by affecting acidification, chelation, precipitation and redox reactions. These compounds can also act indirectly, through their effects on microbial activity, by changing physical and chemical properties of rhizosphere, root growth pattern and soil organic contents [122,123,125,127–132].

LMWOAs are usually present in soil solution in mM range [127,133], with rhizosphere concentration higher in comparison with bulk soil [134]. However, their amounts and compositions are dependent on plant genotype, plant growth stage, plant transpiration rate, growing season and environmental conditions such as CO_2 , light, pH, temperature, moisture, concentration and chemical form of nutrients and the presence of toxicants [27,63,122,124,127,131,132,135,136]. LMWOAs release by plant roots and their composition vary with environmental conditions and physico-chemical rhizosphere properties. For example, LMWOAs are released at higher rate from roots during oxidative stress, under high level of Pb and iron, or low concentrations of calcium and phosphorus [27,122,127,135]. Indeed, under Pb stress conditions, H^+ -ATPases located at the plasma membrane level become activated resulting in the release of organic exudates from roots [122]. Therefore, LMWOAs are suggested even more important than soil pH, because their release and role vary according to environmental conditions and plant needs [135,137].

3.1. Effect of LMWOAs on Pb speciation and behavior in soil

Typically, rhizosphere consists of soil layer (1–5 mM) surrounding root cylinder [138,139]. By modifying rhizosphere chemistry and processes operating [110,124,125,131,132,140] and supplying both protons and complexing anions, LMWOAs can facilitate Pb desorption from soil components [121,122,141] and Pb-compounds dissolution [135]. Debela et al. [142] reported increased Pb release from pyromorphite, a presumably stable Pb-phosphate [$Pb_5(PO_4)_3Cl$] mineral, in contaminated soils by LMWOAs. Wu et al. [63] stated that LMOWAs introduction into the soil resulted in 20–30 times increased Pb level in soil solution.

Because Pb adsorption on soils decreased at lower pH values [13,19,27,110,143–145], H^+ released by carboxylic acid groups of LMWOAs play a major role in Pb dissolution [110,122,131]. Fest et al. [146] observed highest $CaCl_2$ Pb extractable at low pH. They suggested that Pb solubilization was influenced by local competition between H^+ or Ca^{2+} and Pb^{2+} for solid phase adsorption. Separately or simultaneously, the production of protons, exudates and metabolites released in rhizosphere by roots and microorganisms can modify soil pH by as much as one or two units depending, for instance, on the soil physico-chemical properties, plant type and composition of LMWOAs [122]. However, Pb solubilization in rhizosphere is not always related to decrease in soil pH by LMWOAs. Recently Kim et al. [21,131,132] shows that Pb solubilization in alkaline soil by *B. juncea* was due to increase in soil dissolved organic carbon (DOC) contents and Pb–DOC complex formation.

From another side, few authors concluded that LMWOAs have little or no effect on Pb solubility [63,80,100] due to low metal binding capacity [31] or short half-life (1.5–5.7 days) in soils [147]. Actually, the influence of root exudates on Pb solubilization depends on soil physico-chemical conditions and microbial activity [44,79,140,148]. For example Kim et al. [21,131,132] demonstrated that root exudates from *B. juncea* and *H. annuus* increased soluble Pb contents in alkaline soil, but decrease in acidic. In addition, LMWOAs effectiveness is also the function of plant species [122].

3.2. Effect of LMWOAs on Pb plant uptake and accumulation in different parts

LMWOAs can influence Pb uptake by plants and translocation to aerial parts [31,39,125]. Liu et al. [79] reported that citric acid significantly increased Pb root uptake by accumulating and non-accumulating *S. alfredii* plants, but increased Pb translocation to shoot was observed only for accumulating *S. alfredii*. Several other authors reported increased Pb translocation with LMWOAs [110,149]. The mechanism behind this increased Pb translocation to shoot tissues by LMWOAs is still unknown. However, it is proposed that LMWOAs-induced increased Pb translocation in hyperaccumulator plants might be due to the presence of carrier proteins, which bind and translocate Pb to shoot tissues [150–152]. The Pb hyperaccumulator plants secrete large quantities of LMWOAs to mobilise Pb in soil for increased uptake [150]. Inside the plant roots, carrier proteins bind this Pb and translocate to shoot tissues. In this way, the LMWOAs increase Pb translocation to shoot tissues in combination with carrier proteins.

LMWOAs effect on Pb uptake and translocation in some plant species is shown in Table 2. Biodegradation of LMWOAs could reduce their influence on Pb solubility [80,153]. However, interaction with Pb can reduce LMWOAs biodegradability [12]. Anyway, Liao et al. [154] presented no significant LMWOAs effects on Pb uptake by *Lactuca sativa*. Chen et al. [110] reported that citric acid had no significant effect on plant uptake or translocation. Some authors reported that LMWOAs effect on Pb uptake and translocation is less significant compare to synthetic chelates like EDTA [2,39,110,155]. Shen et al. [80] reported for different chelating

Table 2
Effect of citric acid on Pb uptake, translocation and toxicity to plants.

CA (mM)	Pb	Duration (days)	Crops	Uptake	Trans.	Effects	Ref.
1.2	2.4 mM	7	<i>Z.elegans</i>	NM	↑ 1.2	No effect on Root and shoot length	[203]
2.4		7			↓ 1.2	↑ shoot length	
4.8		7			↓ 1.1	No effect on Root and shoot length	
2.4	4.8 mM	7			↑ 1.3	↓ Root length	
4.8		7			↓ 1.1	↓ Root length	
9.6		7			↓ 1.16	↓ Root length	
5	254 µg/l	4	<i>B.juncea</i>	NM	↑ 8	No effect on shoot dry weight	[207]
	35 µg/l	4			1	No effect on shoot dry weight	
	254 µg/l	4			1	↓ Shoot dry weight	
	35 µg/l	4	<i>L.perenne</i>		1	No effect on shoot dry weight	
5	1015 mg/kg	10	<i>S.alfredii</i>	2.4	1.4	↓ Shoot dry weight	[79]
5	1331 mg/kg	7	<i>B.juncea</i>	1	1	↓ Plant dry weight	[122]
5	25 mg/kg	14	<i>Raphanus sativus</i>	↓ 2.4	↓ 1.3	↓ Pb toxicity	[110]
5	44.2 mg/kg	7	<i>Z.mays</i>	NM	↑ 1.5	↓ Shoot and root dry weight	[149]
		14			↓ 1.7	↓ Shoot and root dry weight	
		7	<i>P.vulgaris</i>		↑ 3.5	↓ Shoot and root dry weight	
		14			↓ 1.1	↓ Shoot and root dry weight	
5	58 mg/kg	5	<i>S.alfredii</i>	↑ 1.1	↑ 1.1	↓ Shoot, root, leaf and stem DW	[39]
8		5		↑ 2.2	↑ 1.2	↓ Shoot, root, leaf and stem DW	
1.25	7500 mg/kg	14	<i>S.drummondii</i>	↑ 5	↑ 2.4	↓ shoot length, ↓ chlorophyll a	[94]
2.5		14		↑ 13	↑ 2.8	↓ shoot length, ↓ chlorophyll a	
5		14		↑ 4	↑ 2.1	↓ shoot length, ↓ chlorophyll a	
10		14		↑ 3	↑ 1.7	↓ shoot length, ↓ chlorophyll a	
62.2	50 mg/kg	21	<i>N.tabacum</i>	NM	↑ 1.2	↓ Shoot dry weight	[140]
62.2	300 mg/kg	21			↑ 1.2	↓ Shoot dry weight	
62.2	600 mg/kg	21			↑ 1.1	↓ Shoot dry weight	

NM, not mentioned; Trans, translocation; ↑ indicates increase and ↓ indicates decrease.

agents (in decreasing order) effectiveness for stimulating Pb accumulation in *Brassica oleracea* shoots: EDTA > HEDTA > DTPA » citric acid.

3.3. Effect of LMWOAs on Pb-induced toxicity to plants

First, LMWOA addition can differently influence plant development, depending on plant and LMWOA nature. A positive LMWOA (especially citric and oxalic acid) influence on plant growth was reported by Duarte et al. [156] for tobacco shoots. Luo et al. [149] observed no significant effects of citric acid application on dry matter production of *Z. mays* and *P. vulgaris*. Evangelou et al. [140] reported toxic effects of citric acid application on *N. tabacum* plants. Türkoğlu [157] also reported significant decrease in mitotic index by citric acid in *Allium cepa* L. and Shahid et al. [2] reported that high citric acid concentration (>mM) induced genotoxicity to *V. faba*.

By chelating Pb in rhizosphere or apoplastic spaces, LMWOAs could change its phyto-toxicity and can prevent its entry into the symplast. In this way, Pb tolerant species maintain low shoot Pb level by restricting its uptake and translocation over a wide range of external concentrations. However, variation in the rate and composition of root exudates by different species is still under debate with respect to their role inside plant and in rhizosphere under Pb and other stress conditions. Chen et al. [110] concluded that Pb toxicity decrease to radish in presence of citric acid was due to change in Pb speciation in soil solution and inside the plant. Several authors concluded that as affinity of citric acid for Pb is low, only high concentrations of this chelate can modify phyto-toxicity [2,106]. LMWOAs effect on Pb-induced toxicity in some plant species is shown in Table 2: according to plant and LMWOAs types, contrasted effects were observed on Pb-induced phyto-toxicity.

4. HSs

According to Evangelou et al. [140,158], natural organic molecules could be used as an alternative to potentially toxic and high-cost synthetic chelating agents. This is due to large specific surface area, high CEC, low cost and wide spread availability [52].

DOM consists of humic and non-humic substances; HSs consist of humic acids (HAs), fulvic acids (FAs) and humin. HA is the fraction of HSs that is not soluble in water under acidic conditions (pH < 2) but is soluble at higher pH, with average molecular weight of 2000–3000. FA is the fraction that is soluble at all pH values, with average molecular weight less than 1,000. These substances have similarities but vary greatly from one another (according to origin) with respect to molecular weight, molecular size, substructures and functionalities, depending on the nature and sources of organic matter, from age and functional groups. Functional groups include COOH, phenolic, enolic and alcoholic OH, quinone, hydroxyquinone and lactone. Chelation by neighboring carboxyl and phenolic groups give them an exceptional capacity for Pb complexation [144,159–165]. The protonation or de-protonation of functional groups in response to changes in soil pH influence the efficiency of HSs to form complexes with Pb and other metals [102]. These functional groups undergo protonation at low pH, while deprotonation is generally favored at high pH [166]. Due to lower molecular weight, higher oxygen contents, number of functional group and exchange capacity, FAs are more reactive than HAs [167,168].

4.1. Effect of HSs on Pb speciation and behavior in soil

Humic substances are involved in many soil processes such as solubilization/precipitation, sorption/desorption and reduction/oxidation. These processes affect soil chemistry by changing soil pH, CEC, redox status, buffering capacity, fixation/aging, chemical form of organic and inorganic ligands, mycorrhization and competing ion concentration, which in turn modify Pb mobility, bioavailability and toxicity [18,19,45,143,161,169–173]. HSs react with Pb to form complexes, thus influence the concentrations of free Pb²⁺ and labile Pb-complexes and their subsequent mobility and biotoxicity in soil and target organisms [163,170,174,175].

Humic substances may exist in solid or aqueous phases (either dissolved or suspended form) depending on environmental conditions [102,176]. In the dissolved form, HSs have the ability to form Pb-complexes. When present as an organic solid phase, HSs provide

Table 3
Effect of HSs on Pb uptake, translocation and phyto-toxicity.

HS (mg/kg)	Pb (mg/kg)	Duration (days)	Crops	Uptake	Trans.	Effects	Ref.
10	200	12	<i>L. minor</i>	NM	↑ 1.9	↑ Chlorophyll contents, growth rate	[196]
20	200	12			↑ 3.3	↑ Chlorophyll contents, growth rate	
40	200	12			↑ 2.8	↑ Chlorophyll contents, growth rate	
80	200	12			↑ 1.8	↑ Chlorophyll contents, growth rate	
160	200	12			↓ 1.6	↑ Chlorophyll contents, growth rate	
125	125	60	<i>B. napus</i>	↓ 2.6	↓ 1.1	NM	[187]
500	500	60		↓ 1.7	↑ 1.1		
1000	1000	60		↓ slightly	↓ 1.6		
2000	2000	60		↓ slightly	No effect		
140	150	28		↑ 1.4	↑ 1.4	↑ Shoot and root dry weight	
280	150	28	<i>T. aestivum</i>	↑ 1.7	↑ 1.7	↑ Shoot and root dry weight	[191]
560	150	28		↑ 1.9	↑ 2.6	↑ Shoot and root dry weight	
53,000	122	55	<i>Z. Mays</i>	NM	↑ 1.6	↑ Dry weight	[193]
400,000	6703	112	<i>A. capillaris</i>		↑ 10		

NM, not mentioned; Trans, translocation; ↑ indicates increase and ↓ indicates decrease.

a surface for Pb adsorption from the aqueous system. However, inconsistency exists in the literature on the role of HSs towards Pb bioavailable fraction in soil [56,162]: both a decrease in Pb bioavailable fraction [45,108,177,178] and an increased bioavailable fraction in the presence of HSs [56,179,180] were observed.

According to the free ion activity model and its recent extension biotic ligand model, DOM could decrease Pb bioavailable fraction in soil medium by binding Pb^{2+} ions and thus reducing their available concentration [56,102,181]. Evangelou et al. [158] observed that Pb bioavailable fraction and other metals increase in HAS amended soils due to metal-HS complexes formation, which are taken up by plants with more ease than free metal ions. Halim et al. [159] reported that 2% HA addition reduced extractability of the soluble and exchangeable Pb forms. Farrell et al. [182] observed that soil solution Pb concentration decreased in an acidic contaminated soil after the HSs (as compost) application. Botero et al. [161] showed that HSs have high complexing affinity for Pb compare to essential nutrients.

On the other side, some reports indicate that HSs increase Pb bioavailable fraction in soil. Khan and Scullion [183] reported increased extractable Pb with increasing HA concentrations. Laborda et al. [184] also stated that HAS contribute to Pb mobilization. Dai et al. [185] found that DTPA-extractable Pb content in contaminated soils correlates with organic matter contents in soils. However, the fraction of Pb solubilization by HSs varies considerably between soils. Clemente and Bernal [186] reported that HSs addition to the acidic soil cause less solubilization of Zn and Pb compare to calcareous soil. Zeng et al. [56] described a positive correlation between HSs content and EDTA-extractable Pb content in a contaminated soil. Gharbi et al. [44] observed increased mobility of organically-bound Pb in soil.

These contrasted results could be due in particular to various HSs nature and conformation changes in function of physico-chemical media conditions. HSs-induced Pb solubilization in soil also depends on chemical and structural characteristics of HSs [163]. Key factors controlling metal solubilization efficiency of HSs includes reaction constant [102], HSs co-application [187], different humification stages of HSs [188], molar mass or size of HSs [189], dilution factor [102] and pH [190].

4.2. Effect of HSs on Pb plant uptake and accumulation in different plant parts

The role of HSs towards Pb plant uptake is very complex due to its heterogeneous structure and polymerization degree [163]. HSs form soluble Pb-HS complexes with free Pb ions [56,87], which could be readily taken up by plant roots [158,191]. Therefore, application of HSs can increase Pb plant uptake. Halim et al. [159]

stated that HAS soil amendment accelerates Pb phytoremediation. Bandiera et al. [192] showed HA tendency towards enhanced translocation of Pb to the shoot. Salati et al. [193] reported that Pb uptake was favored by HSs presence. Khan and Scullion [183] also reported increased plant Pb concentrations with increasing concentrations of HA in solution. Higher Pb bio-uptake by the *Chlorella kesslerii*, *Chlorella vulgaris* and *Stephanodizcus hantzschii* has been reported in the presence of the Suwannee River fulvic acid or riverine organic matter [178]. Ho et al. [194] also confirmed higher accumulation of Pb in kenaf roots after the application of organic fertilizer using scanning transmission electron microscope X-ray microanalysis. Evangelou et al. [158] reported HA addition increased metal concentration in shoots of *N. tabacum*. Bandiera et al. [192] reported dose dependent increase in Pb concentration in the shoots of *Raphanus sativus* in response to HA application (0.1 and 1 g kg⁻¹ of soil). Table 3 presents Pb uptake by different plants species in the presence of HSs.

In contrast, Mager et al. [175] reported strong protection against Pb accumulation by increased DOC in soil. Slaveykova et al. [178] reported that Pb uptake by *Pimephales promelas* decreased in the presence of Suwannee River fulvic acid with respect to non-complexed Pb. Kalis et al. [195] stated that application of HA decreased Pb uptake by *Lolium perenne* due to high DOC affinity. Kruatrachue et al. [196] reported that application of HSs decreased Pb uptake by *Lemna minor* only at higher concentrations (>100 mg l⁻¹). Addition of organic fertilizer is also reported to reduce the Pb mobility in soil and ultimately the uptake by plants [197].

These contrasting effects of HSs on Pb uptake by plants depend on their efficiency to form Pb complexes. HSs are reported to form polymer in nutrient solution, which make them very complex and resistant to degradation by microorganisms and other physico-chemical factors of soil [163]. This polymerization phenomenon makes strong and large size complexes with Pb, which are not easily taken up by plants. However, the phenomenon of polymerization varies with their concentration, in addition to physico-chemical factors of soil. Under conditions where this phenomenon does not occur (diluted conditions), HSs makes soluble and mobile complexes with Pb (Pb-HSs), which could enter plants more easily due to small size and thus increased Pb uptake [158,198].

4.3. Effect of HSs on Pb-induced phyto-toxicity

Many studies provide evidence of HSs participation in the development of tolerance against Pb toxicity and several protection mechanisms were proposed. According to Babich Stotzky [199], the mechanism of protection provided by HSs against lead toxicity may have been similar to that of the clay minerals, i.e.,

adsorption to the exchange complex and, therefore, limiting the availability of Pb for uptake by the living organisms. Guerrero et al. [177] reported decreased Pb toxicity in the presence of HSs due to decreased uptake of free metal ions. Khan and Scullion [183] also stated increased plant biomass with increasing HAs concentrations in solution. Kruatrachue et al. [196] reported that HSs application inhibited Pb-induced decreased total chlorophyll contents and growth rate of *L. minor*. Grosell et al. [200] demonstrated protective of DOC as Aldrich humic acid. Mager et al. [175] also reported that strong protection was afforded by DOC against acute Pb toxicity to *P. promelas*, whereas milder protection was observed for *Ceriodaphnia dubia*. However, Bandiera et al. [192] reported phytotoxic effects of higher levels of HA (10 g kg^{-1} of soil) to *R. sativus*. Table 3 summarizes the effects of HSs on Pb-induced phytotoxicity, it can be noticed that HSs can variously modify both Pb transfer and phyto-toxicity. As previously observed for Pb fate in soil and phyto-availability, contrasted results were recorded and could be explained both by media parameters and HS characteristics.

4.4. Use of speciation models to calculate probable application value of organic ligands

In order to compare Pb binding efficiency of above mentioned organic ligands and to calculate their probable application value for solubilization of Pb in soil remediation and risk assessment studies, it is necessary to calculate Pb speciation. In natural media the direct measure of metal speciation is often very complex and time consuming [20]. Recent progress in the development of different speciation models, capable of predicting metal speciation in defined medium, gave further impetus to the research in this area. Although, these speciation models cannot measure exact metal speciation but they give a very useful and rapid measure of probable application value of organic ligands in the appropriate system [2,15,102,151,201]. These models are used in literature to compare the measured and predicted metal speciation or to develop the experimental design [2,151,201]. In the present study, two different speciation models, i.e., Visual Minteq ver. 2.60 and Windermere Humic Aqueous Model VI (WHAM-VI) are used to calculate the Pb speciation in nutrient solution with the macro-elements: 5 mM KNO_3 , 5 mM $\text{Ca}(\text{NO}_3)_2$, 2 mM KH_2PO_4 and 1.5 mM MgSO_4 ; and micro-elements: 9.11 μM MnSO_4 , 1.53 μM ZnSO_4 , 0.235 μM CuSO_4 , 24.05 μM H_3BO_3 , 0.1 μM Na_2MoO_4 and 268 μM Fe/EDTA . Table 5 presents the efficiency of organic ligands to chelate Pb in nutrient solution at pH 7. The speciation models results showed the binding capacity in descending order of $\text{EDTA} > \text{FA} > \text{citric acid}$. Addition of only 5 μM EDTA can chelate more than 90% of Pb in nutrient solution. In case citric acid and FA, the quantities required for 90% chelation of Pb are many fold high compare to EDTA. Based on these calculations, the authors proposed the probable application values of EDTA, citric acid and FA are, respectively, 5 μM , 5 mM and 40 mg l^{-1} .

5. Conclusions and perspectives

Pb behavior in the soil-plant system and its phytotoxicity is greatly influenced by chemical speciation. Organic ligands are capable to modify Pb speciation by forming organo-metallic complexes of varying stability, bioavailability and toxicity. Efficiency of organic ligands to modify Pb behavior and impact greatly depends on their metal binding capacity (Table 4). This binding capacity, in turn, depends on molecular structure, amount and type of functional groups of organic ligands.

Therefore contrasted influences were observed comparing the three different ligands. EDTA forms stable and bioavailable neutral Pb-EDTA chelates inducing: (1) enhanced Pb

Table 4

Binding constants of different organic ligands for Pb (source, default database of Visual Minteq version 2.60 and WHAM VI).

Complex	pK
Pb-citrate ⁻	5.7
Pb-(citrate) ₂ ⁻⁴	6.6
PbEDTA ⁻²	19.7
PbHEDTA ⁻	22.5
Pb-HA (pK ₁)	4.1
Pb-HA (pK ₂)	8.8
Pb-FA (pK ₁)	3.2
Pb-FA (pK ₂)	9.4

pK₁ for COOH and pK₂ for OH group.

desorption/solubilization in soils, (2) increased Pb translocation to shoots with disruption of Casparian strip (3) alleviation of Pb toxicity by binding toxic Pb²⁺ cations. LMOWAs effect on Pb fate or impact is generally attributed to decrease in soil pH and increase in DOC or microbial activity. However, due to relatively low stability and soil microbial activity, Pb-LMWOAs complexes can rapidly dissociate before or after plant uptake and LMWOAs influence on Pb translocation to shoots tissues or phyto-toxicity is therefore relatively weak. Finally, the role of HSs towards Pb solubilization, translocation and toxicity varies greatly due to their complex structure. In addition, physico-chemical properties of soil also influence the effect of organic ligands on Pb speciation. All these influencing parameters induce a wide range of changes in Pb fate and impact in soil-plant systems.

Based on the above mentioned link between Pb speciation and its behavior in ecosystems, it has been proposed that further researches on organic ligands influence should be carried out to optimize the best chelators under applied conditions such as physico-chemical properties of soil, plant type and the extent of Pb pollution and purpose of application. Indeed, complex structure of HSs induces strong difficulties to predict their influence on Pb behavior and use them for industrial applications. Pb leaching to groundwater, high stability in soil and toxicity to soil microbial community are the major concerns against practical EDTA utilization at field scale. Natural LMOWAs role towards Pb translocation or toxicity is not clear and largely depends on plant type; in consequence supplementary studies are needed in order to better understand and manage environmental Pb impact.

Further, different toxicity assessment tests could therefore be carried out under different Pb speciation conditions to optimize the most appropriate and best suited ecotoxicological test under applied conditions. These findings could be further used in genetic

Table 5

Efficiency of EDTA, citric acid (CA) and FA to bind Pb in nutrient solution at pH 7 in nutrient solution using Visual Minteq ver. 2.60 and WHAM VI.

EDTA (μM)	Pb ²⁺ (%)	Pb-EDTA (%)
0	69	0
1	57	18
5	10	86
10	1	99
CA (mM)	Pb ²⁺ (%)	Pb-CA (%)
0.1	66	5
1	41	41
5	7	90
10	1	98
FA (mg l^{-1})	Pb ²⁺ (%)	Pb-FA (%)
1	65	14
5	33	56
10	15	80
40	1	99

engineering studies to improve the techniques for desired results. Similarly some additional organic ligands or polymers could be discovered, which are environmental friendly and are capable to efficiently solubilize and transfer Pb. Most of the studies using organic ligands deal with hyper-accumulator plants, which in most cases have natural Pb and/or Pb-complex detoxification mechanisms. The toxic effects of Pb and/or its complexes and the role of plant detoxification mechanism can be better assessed using a metal-sensitive plant like *V. faba* or *A. cepa*. Therefore, it's necessary to conduct some studies concerning the role of organic ligands towards Pb toxicity and detoxification mechanisms in plant metabolisms using Pb sensitive plants to evaluate their role in risk assessment studies in relation with uptake and speciation. Finally, the role of LMWOAs and HSs has been mainly studied with respect to their effects on rhizosphere chemistry or as absorbent phase in remediation studies. There is very little data available regarding their effects on Pb phyto-toxicity. Therefore, it is necessary to study the toxic nature of different Pb-organic acids complexes and their role in Pb detoxification mechanisms such as antioxidant enzymes activities, phytochelatin and reactive oxygen species induction.

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