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REVIEW

Impact of sludge deposition on biodiversity

Sergio Manzetti[1,2] · David van der Spoel[1]

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Abstract Sludge deposition in the environment is carried out in several countries. It encompasses the dispersion of treated or untreated sludge in forests, marsh lands, open waters as well as estuarine systems resulting in the gradual accumulation of toxins and persistent organic compounds in the environment. Studies on the life cycle of compounds from sludge deposition and the consequences of deposition are few. Most reports focus rather on treatment-methods and approaches, legislative aspects as well as analytical evaluations of the chemical profiles of sludge. This paper reviews recent as well as some older studies on sludge deposition in forests and other ecosystems. From the literature covered it can be concluded that sludge deposition induces two detrimental effects on the environment: (1) raising of the levels of persistent toxins in soil, vegetation and wild life and (2) slow and long-termed biodiversity-reduction through the fertilizing nutrient pollution operating on the vegetation. Since recent studies show that eutrophication of the environment is a major threat to global biodiversity supplying additional nutrients through sludge-based fertilization seems imprudent. Toxins that accumulate in the vegetation are transferred to feeding herbivores and their predators, resulting in a reduced long-term survival chance of exposed species. We briefly review current legislation for sludge deposition and suggest alternative routes to handling this difficult class of waste.

✉ David van der Spoel
david.vanderspoel@icm.uu.se

[1] Uppsala Centre for Computational Chemistry, Science for Life Laboratory, Department of Cell and Molecular Biology, Uppsala University, Box 596, 75124 Uppsala, Sweden

[2] Fjordforsk A.S. Institute for Science and Technology, Midtun, 6894 Vangsnes, Norway

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Introduction

Industrialization and modernization have resulted in a series of complications to the global ecosystem, as the increasing production of waste combined with polluting anthropogenic activities represents a direct threat to biodiversity. Wastewater sludge is a an important part of this problem, as it is difficult to transform and contains a long range of new and emerging pollutants (Manzetti et al. 2014), persistent compounds (Amir et al. 2005; Baker et al. 1980; Birkett and Lester 2002) and poorly degradable components, which are difficult to remove (Arthurson 2008). A series of alternative methods of wastewater sludge treatment exist, however most result in the eventual deposition of treated or untreated sludge in the environment. Forests and marsh-lands have been used for deposition in both Europe and the USA for decades (Forster et al. 1977; Theis et al. 1978; Tullander 1975; Vesilind 1979). The consequences attributed to sludge deposition in the environment include groundwater contamination (Brockway and Urie 1983; Schaider et al. 2014), ecotoxic effects (Speir et al. 2003) and risk of spreading pathogens (Arthurson 2008). The practice has caught legislative attention in recent years with respect to the effects of wastewater on sensitive aquatic environments (Manzetti and Stenersen 2010). Here we provide a thorough review of the life-cycle, the environmental transformation path, and the consequences of deposition of sludge in the environment. The review encompasses a survey of the available information of effects of sludge toxins on the environment, with an emphasis on vegetation and animals. We also consider effects on biodiversity in overstory and understory *biota*. Given the lack of proper alternatives to sludge deposition, a proposed technology for reducing and minimizing dispersion of sludge contaminants in the environment is included.

Contents of wastewater sludge

Wastewater sludge derives from treating wastewater effluents from urban as well as rural communities, and represents the final, dry format of processed wastewater (Eckenfelder and Musterman 1998). This fraction contains a variety of pollutants (Buisson et al. 1984), viruses and microbial populations (Arthurson 2008; Hurst and Gerba 1989; Martins et al. 2004), endocrine disruptors (Nakada et al. 2006), persistent organic compounds such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Table 1), and more importantly, inorganic pollutants such as heavy metals (Cd, Pb, Mn, Cu, Zn and Ni) (Amir et al. 2005; Ščančar et al. 2000) at various concentrations (Table 2). Lazarri and co-workers have shown that occurrence of organic toxins and heavy metals is correlated, that is PAHs associate with mercury, while PCBs associate with lead, cadmium and copper (Lazzari et al. 2000). Sludge deposition induces contamination of groundwater (Speir et al. 2003), accumulation of toxins in vegetation (McLaughlin and Singh 1999; Welch and Norvell 1999), dispersion of fine particulate matter in the environment (Seames et al. 2002) and adverse health-effects on humans and animals (Senesil et al. 1999). It has been pointed out that the transformation and migration patterns of pollutants are important factors in ecotoxicity assessments (Manzetti and Ghisi 2014; Manzetti and Stenersen 2010; Manzetti et al. 2014). New and emerging classes of pollutants, such as persistent organic compounds, endocrine disruptors and drug-metabolites deriving from industrial activity and consumer-products are increasingly being related to health-adverse effects on humans and animals, also in the forms of metabolites (Manzetti and Ghisi 2014; Manzetti et al. 2014). Many of such compounds are present in sewage sludge and, as a consequence of spreading these, long-term effects on the environment are induced including disruption of endocrine processes (Kolpin et al. 2002; Lind et al. 2010; Siglin et al. 2000; Zoeller et al. 2005), adverse effects on nerve system function (Tilson et al. 1990; Wormley et al. 2004), animal and plant growth and development problems (Faustman

Table 2 Levels of heavy metals in soils after deposition of sludge

Heavy metal	Contamination level
Zn^{2+}	58 (mg/kg) (Brockway 1983)
	100–600 (mg/kg) (McBride 2003)
Cd^{2+}	1 (mg/kg) (Brockway 1983)
	2.5–34 (mg/kg) (McBride 2003)
Cr^{6+}	7200 kg/ha (James and Bartlett 1983)
Cr^{3+}	2100 kg/ha (James and Bartlett 1983)
Cu^{2+}	11–179 (mg/kg) (Sanders and Adams 1987)
Mn^{2+}	221–551 (mg/kg) (Sanders and Adams 1987)
Ni^{2+}	5–109 (mg/kg) (Sanders and Adams 1987)

Table 1 Measurements of ecotoxicological content of activated and dry sludge from different areas

Compound	Activated sludge (μg/g)	Location	Dry sludge (μg/g)	Location
PAHs	117.7	Paris, France (Blanchard et al. 2007)	7.52	Thessaloniki, Greece (Mantis et al. 2005)
	8310.2	Beijing, China (Dai et al. 2007)	5.429	Jerez de la Frontera, Spain (Villar et al. 2006)
	130	UK (Stevens et al. 2002)		
PCBs	220	UK (Stevens et al. 2002)	1.31	Thessaloniki, Greece (Mantis et al. 2005)
	620	Thessaloniki, Greece (Katsoyiannis and Samara 2005)	0.04	Catalonia, Spain (Eljarrat et al. 2003)
			2.44	Paris, France (Blanchard et al. 2007)
Heavy metals	1626.3	Thessaloniki, Greece (Mantis et al. 2005)	185.5	Beijing, China (Wang et al. 2006)
	2814.5	Beijing, China (Dai et al. 2007)	32.7	Sevilla, Spain (Álvarez et al. 2002)
Dioxins	83	UK (Stevens et al. 2002)	2.6×10^{-4}	Stockholm, Sweden (Broman et al. 1990)
Pharmaceuticals, pesticides	42	UK (Stevens et al. 2002)	0.796	Barcelona, Spain (Radjenović et al. 2009)

et al. 2000; Gomara et al. 2007; Reichrtová et al. 1999), complications with digestive and nutrient-assimilating processes and reproductive functions (Mendola et al. 2008; Newbold et al. 2009; Wigle et al. 2008) as well as cancer in animals and humans (Arlt et al. 2001; Weyer et al. 2001).

Dispersion of contaminants from sludge

Sludge deposition supplies organic and inorganic pollutants to the soil and vegetation (Brockway 1983; Chaney et al. 1977; Godbold et al. 1988; Hirsch et al. 1993) resulting in the introduction of bio-available forms of heavy metals and persistent organic compounds (POC) to wildlife, which is triggered by the contaminants reaching and accumulating in the stems and foliage of vegetation. Transfer of toxins from sludge to the vegetation induces a slow and long-term contamination of the local and regional food chain (Brockway and Urie 1983; Crête et al. 1987; Glooschenko et al. 1988; Gustafson et al. 2000; Labrecque et al. 1995; Ortiz and Alcaniz 2006) (Fig. 1). The practice of sludge deposition in forests has been discussed since the early 1970s (Chaney et al. 1977; Lindsay 1973; Oliver and Cosgrove 1974; Tullander 1975) and received particular attention when solubilized cadmium was found in soils at concentrations of more than 1 mg/kg while zinc levels were found to exceed 58 mg/kg following deposition of sludge in the soil (Brockway 1983). Cadmium from sludge accumulates in the stem and foliage of plants (Kelly et al. 1979; Lepp and Eardley 1978; Petit and Van de Geijn 1978; Welch and Norvell 1999), while heavy metals (Cu, Pb, Ni) are immobilized and absorbed in soil humus and in the upper layers of the soil by colloids and anions (Brockway 1983). Soil anions and soil colloids play a role in the uptake of nutrients and chemical compounds in plants (Seyfferth et al. 2008). Complexation between heavy metals and soil anions can also result in changes in the rate of uptake of nutrients in vegetative species (Dhillon and Dhillon 2000). Interestingly, in the study of sludge deposition in Michigan State, high levels of heavy elements in the soil were found fourteen months after municipal sludge had been applied (Brockway 1983), delineating the high rate of dispersion from applied sludge to soil. For reference, Table 2 summarizes the levels of heavy metals detected in soils after deposition of sludge from several studies.

Soil chemistry changes from sludge deposition

Deposition of sewage sludge in forests results in a decrease of the soil pH (Brockway 1983), which afflicts the existing species and ecosystems (Pärtel et al. 2004). Small reductions in pH stimulate the growth of acid-tolerant microbial, fungal and herbal species which compete for local nutrients and furthermore reduce the viability of the *plantae* that thrive at neutral and more basic pH (Augusto et al. 2002). This path from changed pH to altered biodiversity to

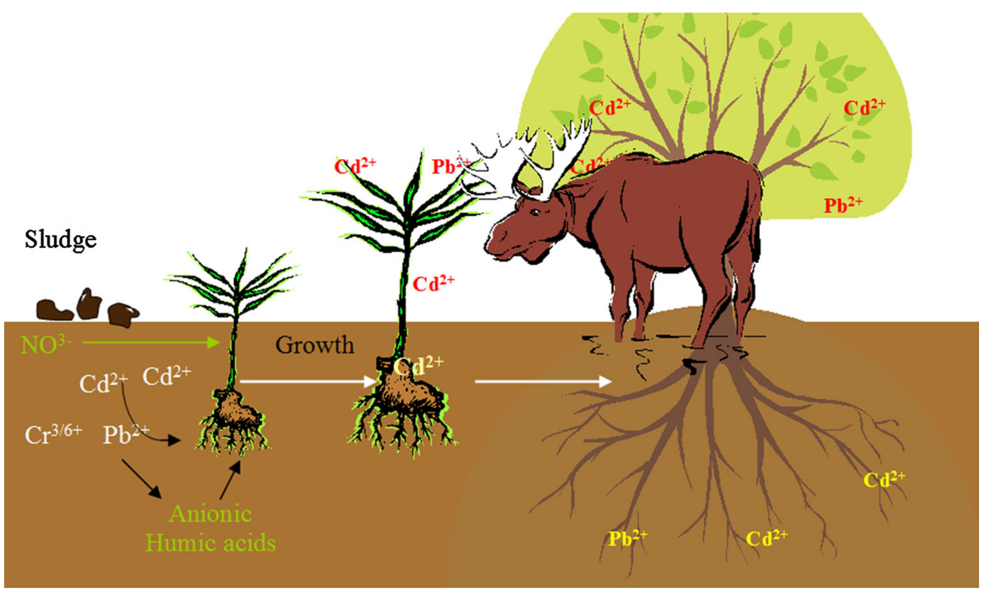

Fig. 1 Schematic illustration of the life cycle of heavy metals from deposited sludge in forest environments. From *left to right*, the growth cycle of plants and trees promotes a gradual accumulation of heavy metals in leaves and stems, where *leaves* represent the highest concentrations of heavy metals, after the roots (Seregin and Ivanov 2001). The nutrition source for animals in highly exposed areas can therefore contain considerable amounts of immobilized heavy metals. *Red* and *yellow texts* indicate bio-assimilated and immobilized heavy metal ions (in complex with enzymes and proteins and miscellaneous organic compounds). Complexation to anionic soil-acids and compounds is reported (Brockway 1983)

reduced nutrient availability represents a modification of the affected ecosystem, which produces and contains key-nutrients to feeding animals (Blum 2008). A lower soil pH also leads to increased solubilisation of heavy metals (Boekhold et al. 1993) contributing to an increased transfer efficiency of the heavy metals to plants, roots and general understory vegetation (Kirkham 2006). The importance of pH was corroborated in a study on the effect of soil composition on bioavailability of heavy metals which was found to be reduced at basic pH (Cesar et al. 2012), as mere solubilization of aggregates of heavy metals with soil compounds at lower pH increases bioavailability.

An increased transfer efficiency of contaminants in understory vegetation has also been reported for PCBs, PAHs and carbon-containing persistent compounds (Hyvärinen and Nygrén 1993; Pankakoski et al. 1993; Rombach et al. 2003), which furthermore alter the chemical composition of the understory soil.

Sludge contains also other pollutants, such as high levels of 4-nonylphenol (Giger et al. 1984), pesticides (Singh et al. 2004) and up to 332 anthropogenic persistent organic compounds (European Commission 2001), all of which are known to accumulate in humans (Calafat et al. 2005; Manzetti et al. 2014) and to have detrimental effects on the environment, animals and plant species (Gray and Metcalfe 1997; Igbedioh 1991; Laws et al. 2000). A sludge-fate study from India found a fertilizing effect of sludge deposition on plant growth due to nitrogen, phosphorous and potassium content, however this was offset by the negative impact on soil and water quality, on health of the local population, agriculture and the environmental in general (Singh et al. 2004). Other environments respond to sludge deposition via soil-chemistry changes. Marsh sites and wet-lands absorb significant quantities of heavy metals, predominantly cadmium, followed by zinc and copper. Cd^{2+} and Zn^{2+} in particular accumulate in roots of plants (Morris 1991; Otte et al. 1991). Marsh environments and other wet-land ecosystems are prone to retain heavy metals for extended periods (Gambrell 1994) and the binding affinity of metals to enzymes in marsh plants is generally known to respect the following order: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+}$. Marsh environments are particularly sensitive to contamination, precisely because of the abundance of water, which solubilizes and disperses contaminants (Kalbitz and Wennrich 1998), making them more bioavailable to the local organisms. Studies on the effects of cadmium and lead contamination in wetlands show that half of the heavy metals from landfill leachates are retained in the wetland (Debusk et al. 1996). Sludge deposition in wetlands, either at artificial sites or natural sites, has also been used as an approach to deposit heavy metals and insoluble waste below the vegetation to deeper soil levels (Scholz 2006). The runoff from wetlands usually connects to other ecosystems, and because solubilized material is easily carried with the run-offs, dispersion into the environment can result from sludge disposal in most cases. The water flow between the upstream and downstream sites in wetlands in turn affects the transformation of toxins, their bio-assimilation and their sedimentation (Lin et al. 2002). The local distribution patterns of chemo-deposition from effluent sources represent a fertilizing potential, where high concentrations of organic matter contribute to the growth of microbial and fungal colonies and rapid destruction of local biodiversity. Similar effects have been observed as a result of the release of untreated wastewater in sub-Saharan Africa (Nyenje et al. 2010). In conclusion, sludge dispersion affects the chemical proportions of the soil, in particular the ionic compounds, potentially resulting in detrimental long-term effects.

Environmental impact

Effects on plants through enzyme-inhibition

Cadmium concentrations in the soil over 0.1 mg/kg from sludge or other sources cause reduction in shoot-elongation, reduced root and shoot dry weights, inhibited growth of spruce trees and also the reduction of crop yields by causing damage to the root tissue (Godbold and Hüttermann 1985; Kelly et al. 1979; Turner 1973). Upon intoxication in vegetation, heavy metals localize in the plants in the roots > leaves > stems > inflorescences > seeds, at respective decreasing levels (Brockway 1983; Seregin and Ivanov 2001). The dominant mechanism of toxicity due to heavy metals in plants is through enzyme inhibition (van Assche and Clijsters 1990). Heavy metal ions are ranked in the following order of inhibition potency (M); Ag^{+}, Hg^{+}, Cu^{2+} $(10^{-7}–10^{-5}) > Cd^{2+}$ $(10^{-6}–3 \times 10^{-5}) > Zn^{2+}$ $(10^{-5}–10^{-4}) > Pb^{2+}$ $(10^{-5}–2 \times 10^{-4}) > Ni^{2+}$ $(10^{-5}–6 \times 10^{-4}) > Co^{2+}$ $(2 \times 10^{-4}–3 \times 10^{-4})$ (Seregin and Ivanov 2001). The most important mechanism of inhibition from heavy metals, in particular Cd^{2+}, Pb^{2+} and Cr^{6+} is due to SH-groups from cysteine residues chelating the ions (Fig. 2), inducing a locking-effect on the protein-fold hampering the dynamics involved in protein function (Hall 2002).

Toxicity occurs also when cellular metabolites and vitamins chelate to heavy metals, particularly between Zn^{2+}/Cu^{2+} and nitrogen-containing metabolites. Zn^{2+} and Cu^{2+} have almost full valence 3*d*- and 4*p*-orbitals in their electronic configuration and readily bind lone pairs from nitrogen atoms covalently. Cd^{2+} and Pb^{2+} in particular have the additional property of displacing Zn^{2+} from biological configurations (e.g. active sites in enzymes) resulting in the deactivation of zinc-dependent enzymes

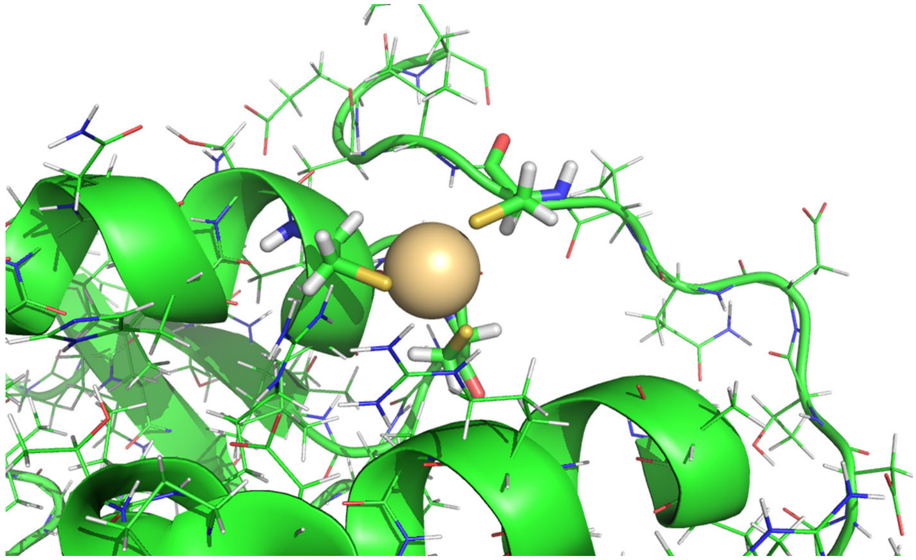

Fig. 2 Chelation of a cadmium ion by the cadmium-sensor protein from *M. Tubercolosis* (Banci et al. 2007). The structure of the binding site shows three cysteine residues (in *stick representation*) that chelate one cadmium ion (*dark yellow sphere*), a binding mechanism similar to the chelation mechanism of heavy metals during enzyme inhibition. Image generated with Pymol (DeLano 2002)

(Seregin and Ivanov 2001), which play important roles in cell-cycle signalling and in cell-switch mechanisms (Egeblad and Werb 2002; Kleiner and Stetler-Stevenson 1999). In biological forms, Zn^{2+} is typically covalently bound to histidine residues in proteins, however in ionic form, Zn^{2+} is a strong *d*-orbital donor for electron rich atoms and can therefore induces uncoordinated covalent bond formation in proteins and smaller biological compounds resulting in a chelation and disruption of their functions. This toxic mechanism may result in the inhibition of the nutrient absorption of plants, inducing detrimental effects on reproduction and causing metabolism failure (Cheng 2003). Heavy metals like Zn^{2+} also induce oxidation stress on glutathione (Ramakrishna and Rao 2013; Wang et al. 2013) leading to impaired growth in e.g. common mullein (Morina et al. 2010). Such effects were found to be aggravated when in addition Cd^{2+} ions were present in a study on maize (Kleckerova et al. 2011).

Heavy metals in soils and ground, at a concentration of 10^{-2} M of Cd^{2+}, reduce the germinating ratio of barley plants by over 50 % (Cheng 2003), and contamination by heavy metals affects several types of plants negatively, including wheat, maize, pumpkin, cucumber and, garlic—by reducing generic root vitality and photosynthesis at both medium and high concentrations (0.2–10 mg/kg) (Cheng 2003). Cadmium intoxication leads additionally to disruption of plant membranes and induces tumorous growth of plant mitochondria, making chloroplasts unable to synthesize chlorophyll (Cheng 2003). Cr^{6+} and Ni^{2+} ions, which are commonly found in wastewater sludge (Knasmüller et al. 1998) have a high anti-proliferating effect on plants resulting from a reduction in chlorophyll concentrations and disruption of protein function in the cells respiratory mechanisms (Cheng 2003). Soil contamination by Cr^{6+} and Ni^{2+} induces mutagenic effects in plants leading to DNA damage affecting natural genetic polymorphism and genetic biodiversity (Knasmüller et al. 1998). Additionally, plants in general are reported to respond to heavy metal contamination by reduction of nutrient absorption, resulting in the inability to uptake nitrogen- and carbon compounds for energy metabolism (Cheng 2003). DNA damage and abnormal gene-expression patterns in plants have also been attributed to Pb^{2+} and Cd^{2+}, affecting in particular the genes coding for alcohol-dehydrogenase resulting in the disruption of RNA and enzyme activities (Cheng 2003). Similar findings have been reported for mercury and other heavy metals in plants (Duan and Wang 1995). Finally, heavy metals negatively affect the genetic diversity of the nitrogen-fixating bacterium *Rhizobium leguminosarum* bv. *trifolii* (Hirsch et al. 1993) that in turn is responsible for nitrogen fixation in e.g. clover (Janczarek et al. 2010). The primary effect of heavy metals on plants is therefore biochemical inhibition and induced cell death due to the chelation effects on the plants' life-sustaining enzyme systems.

The relationship between heavy metals and biodiversity deserves scrutiny in an assessment of the effects of sludge deposition in the environment as well. This connection has not been studied in great depth, in part due to the complexity. One study (Kandeler et al. 1996) shows that the biodiversity in soils at the microbial and enzymatic level is directly diminished after heavy-metal contamination, however inhibiting effects are reported only for functional diversity, and not species-biodiversity. A more recent study reports that a mixture of contaminants in treated sludge, including heavy metals and phosphor organic flame retardants, negatively affects the species composition among macroinvertebrates in German river systems (Stalter et al.

2013). Toes et al. report in a study of bacterial ribosomal RNA that on heavy-metal polluted site the species composition was affected, with Cd and Cu-tolerant microbial species becoming more abundant in comparison to an unpolluted site (Toes et al. 2008). Efforts are underway to determine more systematically to what extent sites are polluted, e.g. by investigating soil nematodes (Zhao and Neher 2013), and also to monitor the effectiveness of sludge treatment (Bafana et al. 2015).

There is also a series of studies reporting on the activity and functions of heavy-metal tolerant plant species, such as the short-rotation willow coppice (Baum et al. 2009; Dimitriou et al. 2006), which proliferate under high levels of heavy metals in soils. These studies offer no systematic comparison with other species or for that sake, with native species from the affected local environments, however they do report on the existence and the characteristics of these species particularly adapted to heavy-metal polluted soils. Proliferation of heavy-metal resistant fungi in cadmium-contaminated soils has also been reported (Weissenhorn et al. 1993) and yet other studies describe differentiation between fungi species of tolerance towards cadmium and lead (Garg and Aggarwal 2011), which all together indicate a selective proliferation of tolerant *biotas* in heavy-metal contaminated soils, thereby indeed modulating biodiversity towards a predominant thriving of heavy-metal tolerant-species. Interestingly, a further study (Schützendübel and Polle 2002) suggests that the role of fungi and microbial populations in the uptake of nutrients in plants - by the interaction with their roots is a critical point affecting biodiversity of plants directly, where heavy-metal tolerant microbial species with alternative rates of nitrogen fixation can affect the nutrient availability for the native vegetation, changing their proliferation potential. The same study (Schützendübel and Polle 2002) reports that different heavy metals chelate nutrients in the soil with varying efficacy, which is in agreement with quantum chemical studies performed by our lab which suggest that lead and chromium bind stronger to for instance vitamin B, than copper, iron or zinc, inducing stable bonds to the vitamin (data not shown).

An ecosystem exposed to heavy metals is prone to direct toxicity effects by biochemical inhibition, but also to a selective development of the local biodiversity dependent on the relative abundance of heavy-metal tolerant and intolerant plant-, fungal and microbial species (Toes et al. 2008; Zhao and Neher 2013). The field of research relating biodiversity to sludge-deposition-induced heavy metal contamination is in direct need of further studies to confirm these connections, as several industrialized countries such as the United Kingdom have heavily polluted soils by sludge deposited heavy metals, with up to 150 mg heavy metals per kg soil (Alloway and Jackson 1991).

Effects on wild life and animals

Sludge deposition provides a source of toxins in addition to the other natural contaminations of the environment. Many forest ecosystems have been contaminated by heavy metals deriving from several sources, including deposited sludge (Amir et al. 2005; Arthurson 2008; Brockway 1983; Chaney et al. 1977; Lazzari et al. 2000; Lindsay 1973; McBride 1995; McBride et al. 1997; Muchuweti et al. 2006; Ortiz and Alcaniz 2006; Ščančar et al. 2000; Seames et al. 2002; Speir et al. 2003; Street et al. 1977; Tullander 1975). These examples of contamination of wild life by persistent inorganic compounds have been shown to affect reindeer, moose, wild boar, squirrel and bear, which graze on trees and vegetation. Intoxication by heavy metals is detected primarily in the kidney and liver of these animals. The most affected species among boreal forests are moose and bear, for which cadmium levels of 4.78 and 6.08 ppm/wet weight kidneys, respectively, have been reported (Medvedev 1999). Among animals tested, reindeer have the highest level of cadmium in muscle tissue while wild boar, bear and moose display the same characteristics of accumulating cadmium in the kidney, liver and lungs (Medvedev 1999). Lead is, in a similar fashion to cadmium, accumulated in the liver, kidneys and lungs and has been found at high concentrations in moose, reindeer and squirrel (Medvedev 1999) (Table 3). Although the report by Medvedev is not directly related to sludge disposition it is instructive because it illustrates that heavy metals are readily taken up through food into higher animals. Further studies from contaminated boreal forests show also high cadmium levels in caribou and muskoxen, with concentrations of 1.9–4.4 ppm for liver and 9.6–33.8 ppm wet weight for kidney (Gamberg and Scheuhammer 1994). Parallel studies on moose from Finland show that some of the metals are transferred from cow to foetus (Hyvärinen and Nygrén 1993). Other species from boreal forests such as wolves have been found to have a high concentrations of PCBs in the liver (Gamberg and Braune 1999) (Table 3). Based on the high levels of toxins in wastewater sludge, deposition of treated and untreated sludge at moderate to high levels represents therefore a considerable risk for the overall viability of forest ecosystem, via the cycle of biotransformation of the heavy metals and persistent organic compounds (Fig. 1).

Inhibitory and stimulating effects of sludge deposition

Fertilizing compounds from sewage sludge, run-off debris and solubilized matter affect the biodiversity of exposed ecosystems starting from microbiological organisms and gradually affect higher order species (Hooper et al. 2012;

Table 3 Levels of heavy metals detected in animals

Animal	Heavy metal	Organ	Levels
Moose	Cd, Cu, Zn	Kidney, Liver	4.78 ppm/w.w., 2.67–232 μg/g, 2.44–128 μg/g (Medvedev 1999)
	Cu	Liver	~50 μg/g (Hyvärinen and Nygrén 1993)
Brown bear	Cd	Kidney	6.08 ppm/w.w (Medvedev 1999)
Caribou	Cd	Liver	1.9–4.4 ppm/w.w (Gamberg and Scheuhammer 1994)
Muskoxen	Cd, Cu	Liver	9.6–33.8 ppm/w.w (Gamberg and Scheuhammer 1994)
			179–300 μg/g (Rombach et al. 2003)
Mole	Cd	Liver	4.48–13.76 μg/g (Pankakoski et al. 1993)
		Kidney	5.31–80.98 μg/g (Pankakoski et al. 1993)
Mole	Pb	Liver	2.05–2.28 μg/g (Pankakoski et al. 1993)
		Kidney	2.09–2.21 μg/g (Pankakoski et al. 1993)
Mole	Hg	Liver,	0.21–0.24 μg/g (Pankakoski et al. 1993)
		Kidney	0.54–0.63 μg/g (Pankakoski et al. 1993)

Martin-Laurent et al. 2001). A reduction of genetic biodiversity in soil samples exposed to sludge deposition has been demonstrated using soil phylotypes, which are DNA-segments that indicate genetic diversity between familiar species (Fig. 3). These studies show a strongly diminished biodiversity of phylotypes at sludge-exposed sites (Martin-Laurent et al. 2001). Modifications of specific DNA-fingerprints in the existing phylotypes (unexpected DNA mutations) result after sludge deposition (Martin-Laurent et al. 2001), indicating a clear effect from the genetic level. This decreased and altered biodiversity may be caused by heavy metal contaminants which induce a reduction in genetic polymorphism in soils (Gao et al. 2010). The reduction of genetic variation in the DNA-pools induces changes in the microbiological populations that in turn affect the ability of the flora and fauna to transform nutrients, as both understory and overstory vegetation are critically dependent on microbial and fungal populations [e.g. for nitrogen fixation (Janczarek et al. 2010)]. This can result in a long-term impact on the biodiversity of exposed ecosystems including forests and loss of biodiversity in itself will in the long run reduce the productivity of the ecosystems (Hooper et al. 2012).

Eutrophication is a critical and additional effect resulting from sludge deposition, which affects the populations and diversity of organisms in the exposed soil (Andres 1999; Parisi et al. 2005). Particular species absorb and metabolize the excessive nutrients supplied by the sludge, while others do not adapt or are outcompeted. The excessive nutrients in the form of sulphates, nitrates and carbon

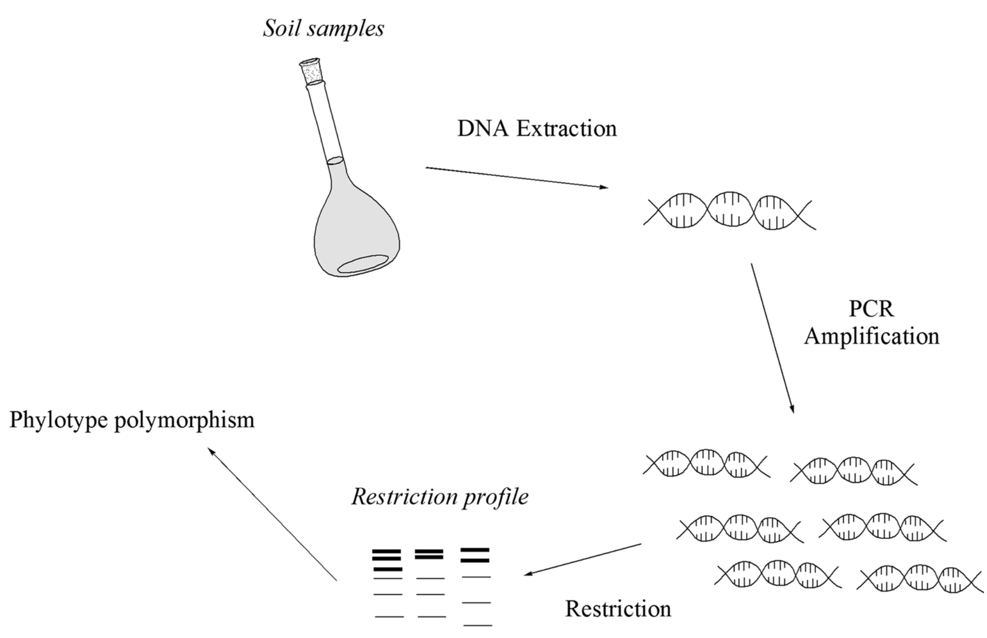

Fig. 3 Phylotype-fingerprinting from soil DNA pools. The process starts from extracted DNA from soil samples, and after PCR and restriction, enzymatic digestions reveal biodiversity from the DNA level through the variation of genetic polymorphism

compounds alter the nutrient cycle in the local ecosystem (Brockway and Urie 1983) and lead to increased concentrations of sulphate and ammonium salts in the soil, which in turn affect the nutrient basis for the whole ecosystem, as observed, for instance, in the Northern United States (Driscoll et al. 2003). This chain of events is known as *nutrient pollution* (Woodward et al. 2012) and has been observed all over the world during the last decades. Nutrient pollution is caused by a range of sources of fertilizing compounds such as air-pollution, water pollution and other sources of anthropogenic origin, including sludge disposal, agricultural and industrial activity (Bobbink et al. 1998; Cheung et al. 2003; Driscoll et al. 2003; Lovett et al. 2009; Nyenje et al. 2010; Ocean Studies Board 2000; Vitousek et al. 2009). The excess quantities of nutrients deriving from pollution or through deposition in the environment results in the chronic accumulation of sulphate and ammonium in plants and soils, causing long-term changes in the soil and water chemistry, ultimately altering biodiversity (Fig. 4) (Lovett et al. 2009). The fertilizing effects of deposited sludge lead to different ratios of S and N levels in the soil (Brockway 1983; Brockway and Urie 1983), which directly affects nutrient availability for the species originally inhabiting the ecosystem (Brockway 1983; Brockway and Urie 1983; Ocean Studies Board 2000; Puckett 1995; Woodward et al. 2012). In Willow plantations on land used for agriculture previously, deposition of sludge may increase biodiversity by the establishment of new species (Fig. 4), which in such cases can be considered a positive result (Baum et al. 2009; Dimitriou et al. 2006; Mirck et al. 2005; Perttu 1998). In natural environments on the other hand it is known that a systematic change in the nutrient composition leads to the gradual extinction of native species that do not thrive in an environment with changed levels of N and S (Hooper et al. 2012; Mooney and Cleland 2001). Eventually the biodiversity of the affected ecosystems is reduced, which is a problem since the first species to disappear are often the rare ones that support vulnerable ecosystem functions (Mouillot et al. 2013). Many of these ecosystems are valuable native habitats for wild life, as well as efficient in absorbing greenhouse gases (Rousk et al. 2013; Sathaye et al. 1995) especially when undisturbed (Clemmensen et al. 2013). Furthermore, intact ecosystems with high biodiversity provide resilience against infections and diseases for humans, animals and plants (Keesing et al. 2010).

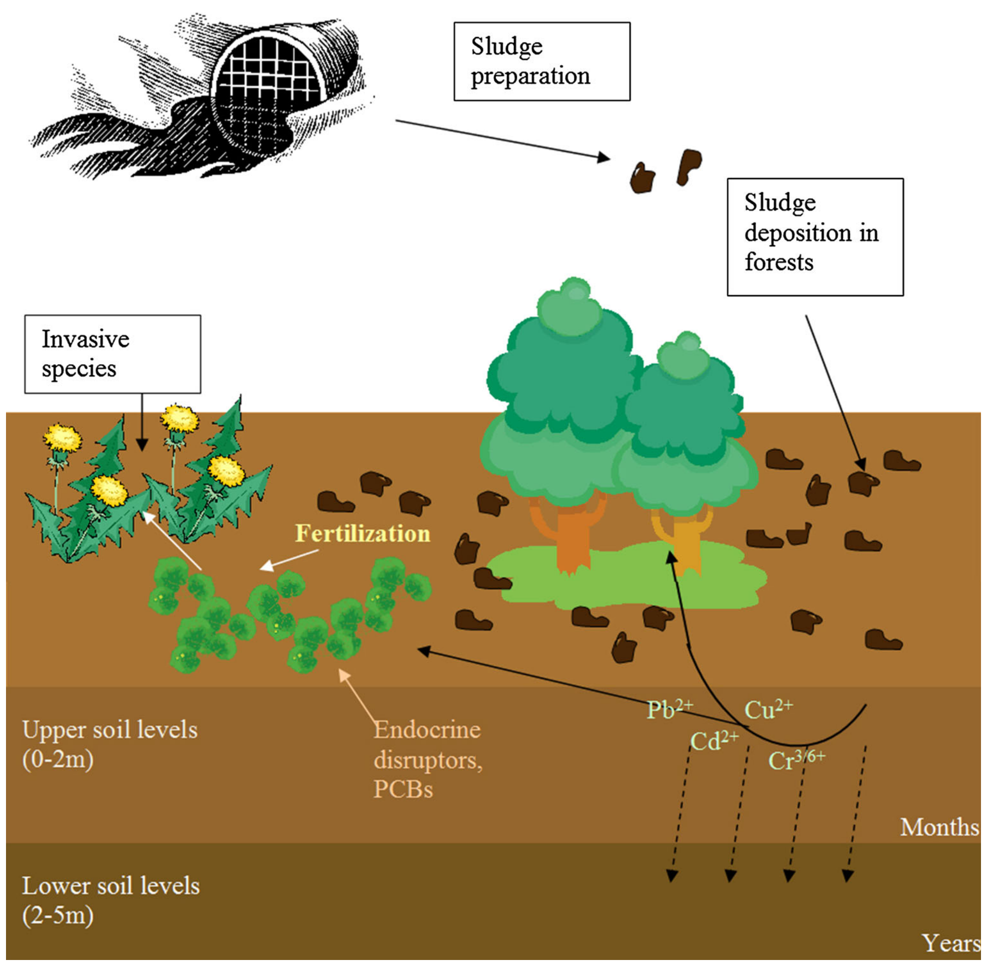

Fig. 4 Paths and periods of transfer of sludge-toxins to the environment. Duration given in weeks, months or years based on literature reviewed. Emerging pollutants denotes antibiotics, pharmaceuticals, hormone-analogues, traffic and industrial pollutants (Manzetti et al. 2014)

The foundation for the local biodiversity is sustained by the fungal population in the understory vegetation (Janczarek et al. 2010) that has been shown to be sensitive to heavy metals from sludge deposition (Hirsch et al. 1993). The response of fungi to changes in N and S levels leads to a chronic change in nutrient availability for the higher order species in the affected ecosystem (Lauber et al. 2008).

Effects from sludge on forest

Sludge dispersion in forests affects plants and trees, via supplying contaminants through the soil matrix, and through direct fertilization of the soil. Fertilization affects the growth and diversity of plants and trees, as particularly elucidated by (Mosquera-Losada et al. 2009, 2012; Rigueiro-Rodriguez et al. 2012). In these studies, Rigueiro-Rodriguez have mapped the effects from dry sludge fertilization with emphasis on birch and pine trees, mapping the growth patterns of the trees over a period of 10 years. The abundance and diversity of the understory vegetation was affected negatively by both sludge- and mineral fertilization (Mosquera-Losada et al. 2009, 2012; Rigueiro-Rodriguez et al. 2012). Fertilization with sludge resulted also in decreased pH levels in the soil, due to higher rate of cation soil extraction. Both birch and pine plantations showed increased soil acidification after sludge fertilization, with a higher effect on the fast growing pines. The affected growth rate of pine trees in turn led to reduced biodiversity in areas rich in *Pinus Radiata*. Plant biodiversity was also reduced by combining dry sludge fertilization and mineral fertilization, where a decrease in the plant diversity, with respect to the non-fertilised (NF) systems was identified by Rigueiro-Rodriguez and colleagues (Rigueiro-Rodriguez et al. 2012). These effects favoured the growth of the sown species, where the taller plants due to stronger growth out-compete the shorter species like *Leguminae* (clovers) further reducing biodiversity. Such biodiversity-altering effects have been reported in another study (Rychtecká et al. 2014).

The effects on overstory from dry sludge fertilization indicate that understory is influenced directly by the composition of the overstory (Rigueiro-Rodriguez et al. 2012). Fertilization of the overstory leads to changes in the understory and affects the vegetation through modifications of resource availability: light, water and soil (Barbier et al. 2008). The availability of light is a major stimulating factor on forest vegetation growth and/or richness (Barbier et al. 2008) and light availability is modulated by the overstory species composition, their leaf sizes, leaf area, canopy form and leaf mass (Coomes and Grubb 2000). As described above, the availability of nutrients becomes directly affected and reduced as the sown species and the sludge-fertilization affects particular species more than others, leading to a reduction in nutrient availability for the native species. This effect is propagated by changes in soil pH, which has been attributed by an additional study on sludge deposition effects on tree growth (López-Díaz et al. 2007). In this study, the pH in the soil was affected significantly by liming and fertilization during the 2 years of the experiments. Liming increased soil pH during the total period, especially when sewage sludge was also applied, due to its higher pH with respect to the soil. The overall growth of tree height and volume increased after fertilization by sludge, contrary to the other results mentioned above (López-Díaz et al. 2007). This different pattern of growth of pine trees was attributed to differences in soil properties, average rainfall during the periods of study and initial pH values in the soil. Boreal forests, which are important subjects for studies of the effects from sludge deposition, are less exposed to heat than the southern European forests studied by Rigueiro-Rodriguez and colleagues (Ferreiro-Dominguez et al. 2011; López-Díaz et al. 2007; Mosquera-Losada et al. 2012; Rigueiro-Rodriguez et al. 2012; Rosa Mosquera-Losada et al. 2010) and may therefore differ considerably in their response to sludge deposition. Currently, no long-term studies on boreal forests from sludge deposition have been published and as the practice of sludge-deposition is still being discussed in Sweden more studies are needed.

Here we have summed up a series of studies and paths of interaction which indicate that artificial fertilization of forests by sludge deposition therefore leads to biodiversity loss starting from the simplest species, however no systematic study evaluating plant and forestal species, biodiversity and proliferation in sludge exposed sites has been conducted by the biological and botanical community. As sludge deposition is a continuous activity in several industrialized nations, and biodiversity in under threat, a set of studies confirming the direct effects of sludge deposition in the environment on biodiversity are required. Additionally, sludge deposition affects and reduces biodiversity in forest ecosystems by diminishing the local resilience to change (Bengtsson et al. 2000) including infectious diseases, which in addition may facilitate the spreading of invasive species (Crowl et al. 2008). The proliferation of invasive species leads to the reduced overall immune-defence of an ecosystem (Mooney and Cleland 2001), as invasive species change the ecosystem's natural nutrient cycles. This is observed when invasive species, which thrive in fertilized (formerly) pristine environments, induce a loss of primary control of the nutrient acquisition of the roots of other plants, and the invasive plants with luxury consumption deprive the environment for minerals from the soil (Chapin III 1980). These effects, deriving from fertilization and sludge deposition (Andres 1999; Andrés and Domene 2005;

Domene et al. 2007), contribute to a disruption of the eco-balance and to extinction of native species (Balvanera et al. 2006; Cardinale et al. 2011; Hooper et al. 2012). Studies on vegetation in the Alaskan tundra report that the *sequence* and dosage of nitrogen, phosphor and carbon species, when disrupted by man-induced fertilization, results in the disturbance of the natural balance of nutrient-processing in the vegetation (Shaver and Chapin 1980) favouring some species over others (Bret-Harte et al. 2001).

Remediation and alternatives

Remediation practices

Modern technologies for remediation of sludge have been developed and these are, to some extent, being applied in wastewater treatment plants (Crain et al. 2000; Fernández-Luqueño et al. 2008; Jakobsen et al. 2004; Jiang 2007; Wong et al. 2002). There are limitations in these technologies however (Bala Subramanian et al. 2010) and the fraction containing heavy metals and insoluble organic parts is often deposited in fragile ecosystems (Arthurson 2008; Tullander 1975). Deposition is in some cases coupled to the cultivation of toxin-absorbing plants, such as short-rotation willow coppice, for the reduction of the detrimental effects (Baum et al. 2009; Dimitriou et al. 2006; Mirck et al. 2005). However, the number of such cases is limited and after decades of sludge deposition in the USA (Forster et al. 1977; Theis et al. 1978; Vesilind 1979), Sweden (Tullander 1975) and other European countries (Bridle 1982), concerns have arisen about the long-term effect of sludge deposition on soils and fertile grounds in forests floors, vegetation areas and plantation systems (Blanchard et al. 2007; Pempkowiak and Obarska-Pempkowiak 2002). Affected ecosystems include pine and birch plantations (Brockway 1983; Chaney et al. 1977; Chubin and Street 1981; Lindsay 1973; Street et al. 1977), agricultural crops (Duarte-Davidson and Jones 1996; McBride 1995; Nicholson et al. 2003), and other habitats, where dispersed sludge results in the accumulation of toxins in the food chain and in the environment in general (Manzetti and Ghisi 2014; McLaughlin and Singh 1999; Seames et al. 2002; Welch and Norvell 1999). This may cause long-term environmental problems for modern society and impact health (Arthurson 2008; Auriol et al. 2006; Manzetti and Ghisi 2014; Ternes et al. 2004).

Legislative aspects

Alternatives to sludge deposition have been discussed, including incineration, composting, land-filling and other forms of transformation, as prompted by the European Commission (European Commission 2001). This has not led to concrete changes in the current practice of sludge deposition in the environment or in agriculture, however. The practice raises a series of questions that need to be considered given that sludge represents a continuous supply of chemically and biochemically interfering compounds to the environment (Hickey and Kittrick 1984) (Fig. 4), with direct consequences for human health if used in agriculture (Muchuweti et al. 2006). This practice should therefore be classified as an unsustainable form of waste disposal (Arthurson 2008), and technologies like electrochemical metal-extraction, precipitation and more extensive treatments of sludge need to be considered for all forms of sludge deriving from wastewater treatment plants. This is also seen in an earlier assessments of the practice of dispersing sludge in the environment, which indicates that EU authorities tend to favour a gradual abolition of the practice and to subject the sludge to incineration for energy recovery over the eventual application on confined land, wherever possible (Davis 1996). As mentioned previously dispersed municipal sludge in forests leaves entities of sludge which are not merged with the soil more than a year after application (Brockway 1983). This introduces a series of questions on the generic environmental “hygiene” and prioritization by legislative entities. Sludge dispersion in forests has been associated with the movement of nitrate species that reached groundwater (Brockway and Urie 1983) and has led to a proposition to enhance the legislative measures to prevent adverse effects on soil microbial communities in order to protect soil fertility (McGrath et al. 1995), as the role of microbial cultures in nitrogen fixation and soil chemistry processes is crucial for ecosystem development, maintenance and biodiversity (Wertz et al. 2006). Current legislation and regulations may be ineffective for reducing the ecological damage represented by sludge deposition in forests. A recent survey of the deposition of sewage sludge and composted sludge shows that after some 40 years the maximum allowed amounts for deposition have been surpassed at the exposed sites for sludge deposition, and that a *doubling* of the international toxicity equivalent (I-TEQ) budget for polychlorinated dibenzodioxins and dibenzofurans and a *threefold* increase for dioxin-like PCBs in soils have been granted in Germany in order to dispose of the growing amounts of municipal sludge (Umlauf et al. 2011). The toxins deriving from deposited sludge have also been shown to affect cultivated agricultural products (Chaney et al. 1978; Fent 1996). A review of legislations from 2001, 2002 and 2005 (European Commission 2001, 2002, 2005) shows that the current regulations do neither consider biodiversity-reducing effects from sludge deposition, nor the contamination of the forest and disruption of wild life nutrient cycles. To maintain and restore vital ecosystems is a prerequisite for humanity to thrive and indeed the reduction of biodiversity is one the largest threats to society (Rockstrom et al. 2009). It would therefore seem prudent to require that a long-termed legislative approach is introduced to separate sludge from the environment and to prevent further introduction of toxins (Manzetti et al. 2014).

Alternative approaches to sludge transformation

An alternative to sludge deposition in the environment is the organized confinement of short-rotation mono-plantations; an extension of a practice applied particularly in Sweden (Dimitriou et al. 2006; Ebbs et al. 1997; Lepp and Madejón 2007). "Sludge-processing certificates" could be granted to farmers for cultivating monocultures for transformation of treated sludge at sites that are isolated from nearby forests and wild life habitats, as well as groundwater systems. Such certificates could be implemented to subsidize farmers to process allotted amounts of treated sludge per year, with follow-ups in the form of yearly soil-sample analyses to determine the fate and transformation of heavy metals and persistent organic compounds (Fig. 5). The granting of sludge-certificates should be limited to plantations that are situated at minimum distances from agricultural sites for food production, or confined by artificial boundaries of sand, such that diffusion and migration of persistent compounds away from the specialized plantations is prevented. Periodic breaks of 5-10 years could be imposed to pause the sludge-transforming activity, so that the local soils are to an extent allowed to further transform the doses of toxic compounds deposited from the sludge-transformation activity before continuation. Extraction via a sink structure with a toxin tank below the field could further facilitate the confinement of these toxins (Fig. 5). Sludge-certificates could also be an alternative way of supporting the critically important agricultural sector. An agreement on sludge-certificates could in other words protect forests and other ecosystems that are exposed to toxins and biodiversity-disrupting fertilization effects deriving from deposited sludge. As a result, wildlife would be protected from exposure to contaminated vegetation, waters and soils.

Conclusions

The fate of the global ecosystem ultimately depends on the climate, preservation of natural habitats and ecosystems and, as covered in this review, a proper transformation of waste and end products. A generic rewriting of the legislation to prevent sludge deposition in forests and other sensitive ecosystems is needed in order to meet the extensive challenges that the international community is facing. The practice of sludge deposition in the environment has curiously been defended, for instance in Sweden, for giving short-term gains in removing sludge from its sources at wastewater plants (Perttu 1998), and transforming these with specialized plantations (Baum et al. 2009; Dimitriou et al. 2006; Mirck et al. 2005; Perttu 1998). However, the short-term gains do not represent a true removal of toxins from the environment but rather a transformation from one phase to another, where the colloidal forms from sludge are biologically converted to bio-assimilated forms, which remain in the soil as bioavailable contaminants.

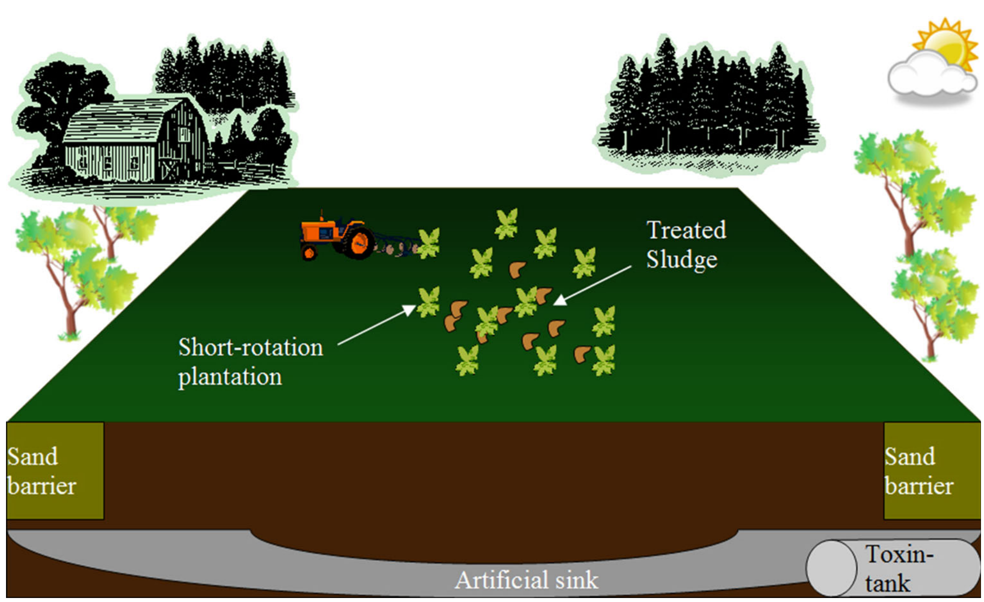

Fig. 5 A proposed rationale for sustainable sludge-transformation. Specialized farms are paid to cultivate short-rotation plantations that absorb and transform sludge. Sand barriers are applied to confine the activity to the plantation, and protect water and neighbouring ecosystems. An artificial sink is constructed to accumulate persistent toxins in a toxin tank, which can be collected yearly for industrial recycling and extraction of metals

Sludge deposition in the environment is a practice of waste handling with long-term impact on soil microbial populations, vegetation and animal proliferation ultimately affecting ecosystem viability. It represents a supply of toxins and fertilization to forests, marshlands and other sensitive environments. Although few measures are taken to prevent deposition of sludge in the environment procedures to remove heavy metals from sludge, through the use of plantations and remediating crops that specifically bind heavy metals from sludge at high concentrations, have been applied. This approach, which leads to the bio-fixation of heavy metals in plants and in soil, does not result in the complete removal of persistent toxins and heavy metals. The fertilization effects from sludge as well as the direct contamination of wild life habitats resulting in intoxication of food chains should be targeted by new legislation governing sludge handling. This should particularly take into account the effects caused by pollutants during the transfer from species to species, such as intoxication of foetuses (Hyvärinen and Nygrén 1993), reduction in reproductive potential (Ferm and Layton 1981) and other long-term effects on biodiversity and animal proliferation (Duarte-Davidson and Jones 1996).

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