ANNEX 5: RBs EFFICIENCY

There are relatively few academic articles on the effectiveness of reed beds on the content of various pollutants, as the topic is new. In reviewing the literature, one can rely on a small group of experts. Most experts are primarily concerned with heavy metals and their content in the final product (biosolids) and the quality of the final product in general - whether it meets the standards for agricultural land disposal. TS and TVS were also reviewed in terms of dry matter volume, hazardous substances, biodegradability and mineralization, and micropollutants and pathogenic organisms. Reed beds have proven effective in removing, decomposing, and accumulating different types of pollutants. A literature review is gathered in ANNEX 7.

The chemical composition of sludge is determined by the amount of total solids, volatile solids, grease and fats, proteins, and nutrients (nitrogen, phosphorus, and potassium). Table 1 presents a typical composition of primary and secondary sludge.

Table 1: Characteristics of primary and secondary sludge[1]

PARAMETER	PRIMARY SLUDGE	SECONDARY SLUDGE
TS (%)	3,0-7,0	0,5-2,0
VS (% TS)	60-80	50-60
Nitrogen (N, % TS)	1,5-4-0	2,4-5,0
Phosphorus (P_2O_5, % TS)	0,8-2,8	0,5-0,7
Potasium (K_2O, % TS)	0-1,0	0,5-0,7
pH	5,0-8,0	6,5-8,0

Solids characteristics that affect land application suitability include organic content, nutrients, pathogens, metals, and microcontaminants. Although several organic and mineral constituents in the sludge may have fertilizing characteristics, others, as metals, trace organic contaminants and pathogens, are associated with sanitary and environmental risks[2].

1.1 Heavy metals

Biosolids disposal in agriculture can lead to an accumulation of heavy metals and organic contaminations in the soil as a result of its long-term application. However, various technologies have been developed in recent years for removing heavy metals from sewage sludge[3]. The best alternative to preserve biosolid quality is the preventive control of trade effluents and illicit discharges into the public sewerage system[4].

Heavy metals (Cr, Cd, Pb, Cu, Ni, Mn, Zn, Fe) in high concentrations can cause health and detrimental environmental effects. Heavy metals concentration in sludge is highly variable, depending on the wastewater source and treatment. Levels are generally lower in domestic wastewater than in industrial

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[2] Ugetti, E. (2011). SEWAGE SLUDGE TREATMENT IN CONSTRUCTED WETLANDS. Technical, economic and environmental aspects applied to small communities of the Mediterranean Region. PhD Thesis, Universitat Politècnica de Catalunya, Barcelona, 199 p.

[3] Kominko, H., Gorazda, K., & Wzorek, Z. (2019). Potentiality of sewage sludge-based organo-mineral fertilizer production in Poland considering nutrient value, heavy metal content and phytotoxicity for rapeseed crops. Journal of Environmental Management, 248(February), 109283. https://doi.org/10.1016/j.jenvman.2019.109283

[4] Tambo, N., Kobayashi, M., Thebault, P., & Haubry, A. (1982). Sludge Treatment and Disposal. Water Supply (Vol. 1). https://doi.org/10.1016/S0167-5648(08)71092-X

wastewaters[5,6,7]. Sludge disposal onto agricultural land is regulated by the European Sludge Directive[8], which controls the land application of sewage sludge according to heavy metals concentrations. Recent regulation proposals are even more restrictive[9].

Several studies show that in general heavy metal concentrations in biosolids are within limits for unrestricted land application[10,11,12], and remained quite unchanged over time[13] or their values are slightly higher due to accumulation through years[14], or decrease concentrations during the drying processes, which could be attributed mainly to heavy metal uptake by the plants[15].

The concentrations of heavy metals generally increased with depth in the vertical sludge profile due to the dewatering and mineralization of organic matter, but in all cases, the concentrations were below the European Union legal limits for agricultural land disposal[16]. Phyto-stabilization may lead to a significant increase of heavy metals within sludge due to volume reduction[17].

As shown in Table 2, a considerable variation in heavy metal concentrations from sludge treatment reed beds is reported in the literature. Nevertheless, in almost all cases heavy metals are below the legal limits[18,19,20,21,22,23]. Thus, according to the results shown in Table 2, biosolids from reed beds might be recycled in soils through agricultural or land restoration applications[24] in terms of heavy metal content in biosolids.

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[5] Ugetti,E. (2011). SEWAGE SLUDGE TREATMENT IN CONSTRUCTED WETLANDS. Technical, economic and environmental aspects applied to small communities of the Mediterranean Region. PhD Thesis, Universitat Politècnica de Catalunya, Barcelona, 199 p.

[6] Shamuyarira, K. K., & Gumbo, J. R. (2014). Assessment of heavy metals in municipal sewage sludge: A case study of Limpopo Province, South Africa. *International Journal of Environmental Research and Public Health*, *11*(3), 2569–2579. https://doi.org/10.3390/ijerph110302569

[7] Liu, J. Y., & Sun, S. Y. (2013). Total concentrations and different fractions of heavy metals in sewage sludge from Guangzhou, China. *Transactions of Nonferrous Metals Society of China (English Edition)*, *23*(8), 2397–2407. https://doi.org/10.1016/S1003-6326(13)62747-8

[8] Council of the European Union (1986). Council Directive 86/278/EEC of 12 June 1986 on the Protection of the Environment, and in Particular of the Soil, when Sewage Sludge is Used in Agriculture. Official Journal of the European Union L 181, 04/07/1986, 6-12.

[9] Environment DG, EU. (2000). Working Document on Sludge 3rd Draft. URL: http://ec.europa.eu/environment/waste/sludge/pdf/sludge_en.pdf (July 2003)

[10] Uggetti, E., Llorens, E., Pedescoll, A., Ferrer, I., Castellnou, R., García, J. (2009a). Sludge dewatering and stabilisation in drying reed beds: characterisation of three full-scale systems in Catalonia, Spain. Bioresource Technology 100 (17), 3882-3890.

[11] Nielsen, S., & Willoughby, N. (2005). Sludge treatment and drying reed bed systems in denmark. Water and Environment Journal, 19(4), 296–305. https://doi.org/10.1111/j.1747-6593.2005.tb00566.x

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[16] Matamoros, V., Nguyen, L. X., Arias, C. A., Nielsen, S., Laugen, M. M., & Brix, H. (2012). Musk fragrances, DEHP and heavy metals in a 20 years old sludge treatment reed bed system. *Water Research*, *46*(12), 3889–3896. https://doi.org/10.1016/j.watres.2012.04.027

[17] Begg, J.S., Lavigne, R.L., Veneman, P.L.M., 2001. Reed beds: constructed wetlands for municipal wastewater treatment plant sludge dewatering. Water Sci. Technol. 44, 393–398.

[18] Council of the European Union (1986). Council Directive 86/278/EEC of 12 June 1986 on the Protection of the Environment, and in Particular of the Soil, when Sewage Sludge is Used in Agriculture. Official Journal of the European Union L 181, 04/07/1986, 6-12.

[19] Environment DG, EU. (2000). Working Document on Sludge 3rd Draft. URL: http://ec.europa.eu/environment/waste/sludge/pdf/sludge_en.pdf (July 2003)

[20] Module, A. N. O., & Rajasthan, F. O. R. (n.d.). *Faecal Sludge and Septage Management*.

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[23] Kominko, H., Gorazda, K., & Wzorek, Z. (2019). Potentiality of sewage sludge-based organo-mineral fertilizer production in Poland considering nutrient value, heavy metal content and phytotoxicity for rapeseed crops. Journal of Environmental Management, 248(February), 109283. https://doi.org/10.1016/j.jenvman.2019.109283

[24] Kołecka, K., Obarska-Pempkowiak, H., & Gajewska, M. (2018). Polish experience in operation of sludge treatment reed beds. Ecological Engineering, 120(June), 405–410. https://doi.org/10.1016/j.ecoleng.2018.06.022

Table 2: Concentration of heavy metals in mg/L and wetland values in mg/kgTS[25].

SYSTEMS' LOCATION		Cr	Ni	Cu	Zn	Cd	Pb	REFERENCE
Buckland, USA	Influent	0,14	0,82	11	3	-	0,5	Begg et al. (2001)
	Wetland	42	23	1906	684	3	154	
-	Wetland	-	-	215	1836	12	341	De Maeseneer (1997)
USA Fort Campbell	Wetland	29*	14*	408*	444*	8	66*	Kim and Smith (1997)
Darzlubie, Poland	Wetland	22	67	28	871	2	31	Obarska-Pepkowiak et al. (2003)
Rudkøbin, Denmark	Wetland	39-99	-	260-470	410-1100	-	-	Nielsen (2003)
Oratoio, Italy	Wetland	40-73	28-52	383-467	1108-1357	<6	93-121	Peruzzi et al. (2007)
Alpens, Spain	Influent	35,8	27,9	227	348	0,41	4,29	Uggetti et al. (2009a)
	Wetland	55	30	390	550	0,6	52	
St Boi de Lluçanès	Influent	36,4	50,2	138	609	0,66	1,99	Uggetti et al. (2009a)
	Wetland	50	36	160	530	0,7	43	
Seva, Spain	Influent	52,1	25	232	897	0,76	0,95	Uggetti et al. (2009a)
	Wetland	60	40	230	690	1	80	
Law thresholds		-	300-400	1000-1750	2500-4000	20-40	750-1200	Council Directive 86/278/EEC
Law thresholds (proposal)		800	200	800	2000	5	500	Environment DG, EU, 2000

*Average from different depths.

Experience has shown that the quality of the final product concerning heavy metals, hazardous organic compounds and pathogens after ten years of treatment make it possible to recycle the sludge residue to agriculture[26, 27].

1.2 Micro-pollutants

Micropollutants are inorganic and organic substances that can negatively affect the environment even at very low concentrations in micro, nano, pico-grams.

Micropollutants (MPs) can be defined as anthropogenic chemicals that occur in the (aquatic) environment well above a (potential) natural background level due to human activities but with concentrations remaining at trace levels (i.e., up to the microgram per liter range). Thus, MPs are defined by their anthropogenic origin and their occurrence at low concentrations. Thousands of chemicals fall into this category. MPs can consist of purely synthetic chemicals, such as strongly halogenated molecules (e.g., fluorinated surfactants), or natural compounds such as antibiotics (e.g.,

[25] Ugetti, E. (2011). SEWAGE SLUDGE TREATMENT IN CONSTRUCTED WETLANDS. Technical, economic and environmental aspects applied to small communities of the Mediterranean Region. PhD Thesis, Universitat Politècnica de Catalunya, Barcelona, 199 p.
[26] De Maeseneer, J. L. (1997). Constructed Wetlands for Sludge Treatment. *Water Science & Technology*, *35*(5), 279–285.
[27] Uggetti, E., Llorens, E., Pedescoll, A., Ferrer, I., Castellnou, R., & García, J. (2009). Sludge dewatering and stabilization in drying reed beds: Characterization of three full-scale systems in Catalonia, Spain. Bioresource Technology, 100(17), 3882–3890. https://doi.org/10.1016/j.biortech.2009.03.047

penicillins) or estrogens. MPs may originate from a wide range of sources (e.g., agriculture, households, transport networks, or industries) and enter water bodies through diverse entry paths. Depending on the source, MP transfer occurs as diffuse (e.g., agricultural land use) or as point source pollution, for which (municipal) WWTPs are relevant examples[28].

The Directive 86/278 has regulated the use in agriculture of residual sludge from domestic and urban wastewater. After 1986, this directive was transposed in the different member state legislation, and currently, the national limit also values some organic micropollutants. Increasingly stringent standards anticipated, especially for emerging organic micropollutants with the upcoming European directive on sludge use on land[29].

Under the Water Framework Directive (WFD), environmental quality standards (EQS) have been established for 45 so-called ‘priority substances’ and eight other pollutants. When the Directive on Environmental Quality Standards was amended in 2013, a watch list mechanism was established to require temporary monitoring of other substances for which evidence suggested a possible risk to or via the environment, to inform the selection of additional priority substances.[30] The surface water Watch List (WL) under the Water Framework Directive (WFD) is a mechanism for obtaining high-quality Union-wide monitoring data on potential water pollutants to determine the risk they pose and thus whether Environmental Quality Standards (EQS) should be set for them at EU level.[31]

Directive 86/278/EEC does not provide any limit values or requirements for organic compounds in sewage sludge. Several national regulations on the use of sludge have added specifications on organic compounds. This is the case in particular of Austria, Denmark, France, Germany, which have all included limit values for some organic compounds in the relevant regulation for the use of sludge. In Sweden, the regulation contains no requirement on organic compounds in sludge; however, restrictions on the concentration of organic compounds in sewage sludge for use in agriculture have been introduced.[32]

Reed beds for mineralization and stabilization of municipal sludge have proven to be useful for reducing micropollutants in municipal sludge. Due to better oxygen conditions, the sludge drying reed-bed with low frequency of sludge input resulted in better removal for organic micropollutants than the sludge drying reed-bed with a high rate of sludge input.[33]

For the sludge treatment, removal efficiencies (Rs) could be determined for up to 34 organic micropollutants. The numbers of Rs in each range (<-30%;-30 to 30%; >30%) are shown on Graph 1 for each sludge treatment.

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[28] Stamm, C., Räsänen, K., Burdon, F. J., Altermatt, F., Jokela, J., Joss, A., Ackermann, M., Eggen, R. I. L. (2016). Chapter Four - Unravelling the Impacts of Micropollutants in Aquatic Ecosystems: Interdisciplinary Studies at the Interface of Large-Scale Ecology. Advances in Ecological Research, Volume 55, 2016, Pages 183-223. https://doi.org/10.1016/bs.aecr.2016.07.002

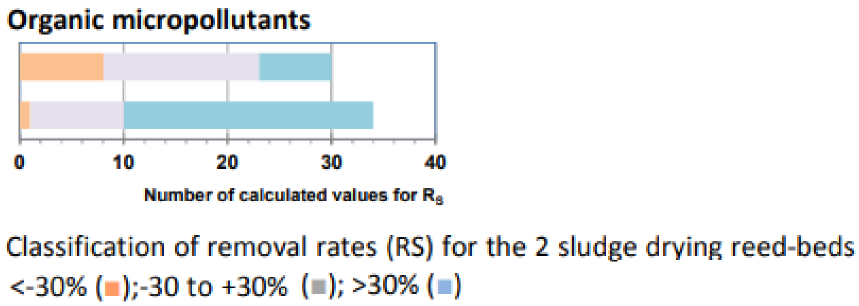
[29] Mininni, G., Blanch, A. R., Lucena, F., & Berselli, S. (2015). EU policy on sewage sludge utilization and perspectives on new approaches of sludge management. Environmental Science and Pollution Research, 22(10), 7361–7374. https://doi.org/10.1007/s11356-014-3132-0

[30] Pollutants from the EU Watch List: a review of their occurrence and water-treatment options

[31] https://publications.jrc.ec.europa.eu/repository/bitstream/JRC111198/wl_report_jrc_2018_04_26_final_online.pdf

[32] https://ec.europa.eu/environment/archives/waste/sludge/pdf/sludge_disposal2.pdf

[33] https://hal.archives-ouvertes.fr/hal-01058679/document



Graph 1: Classification of removal rates (RS) for the two sludge drying reed-beds

Due to right aerobic conditions in the SDRB with low frequency of sludge input (2w_Feed/14w_Rest), this process most of the tested pollutants Rs > 30% due to biotransformation in aerobic conditions (i.e., for estrone, nonylphenol, 7 PAH, 7 PCB, 5 PBDE and three other organics (galaxolide, tonalide, triclosan)). Nevertheless, the Rs values only reached a maximum of 50% for some of the substances regarding PAH, nonylphenol, estrone, galaxolide, triclosan, DEHP. The SDRB with a high frequency of sludge input (2d Feed/14d_Rest) had a majority of Rs values between -30% and +30% (as oxygen was lacking, micropollutants could not be biotransformed, and concentrations were unchanged)[34].

Agricultural application of sewage sludge has been emotionally discussed in the last decades, because the latter contains organic micropollutants with unknown fate and risk potential.

1.2.1 Personal care products

Study »Musk fragrances, DEHP and heavy metals in a 20 years old sludge treatment reed bed system«[35] is the first to report on the fate and concentrations of musk fragrances and DEHP in biosolids accumulated over 20 years in a RBs. Musks are widely used as fragrance ingredients in washing and cleaning agents, in products for personal care and other consumer products. After their use, musks and DEHP are mainly released into the environment due to their inadequate removal by conventional wastewater treatment facilities[36]. Musk fragrances and DEHP are, due to their lipophilic properties, predominantly adsorbed onto the sludge during wastewater treatment[37].

Within the study[38] the effect of biological sludge treatment in a reed bed on reducing the concentrations of the fragrances HHCB, AHTN, OTNE was studied on the bactericide Triclosan. During the twelve months experiment reduction of 20–30 % for HHCB and AHTN, 70 % for Triclosan and 70 % for OTNE were determined under environmental conditions. The reduction is most likely due to degradation, as volatilization, uptake into plants, and leaching are insignificant.

The highest concentrations of the polycyclic musk compounds HHCB and AHTN were determined at the beginning of the project, which is 11.000 ng g−1 (dry weight) HHCB and 2.250 ng g−1 (dry weight)

[34] https://hal.archives-ouvertes.fr/hal-01058679/document
[35] Matamoros, V., Nguyen, L. X., Arias, C. A., Nielsen, S., Laugen, M. M., & Brix, H. (2012). Musk fragrances, DEHP and heavy metals in a 20 years old sludge treatment reed bed system. *Water Research*, *46*(12), 3889–3896. https://doi.org/10.1016/j.watres.2012.04.027
[36] Mie`ge, C., Choubert, J.M., Ribeiro, L., Euse`be, M., Coquery, M., 2008. Removal Efficiency of Pharmaceuticals and Personal Care Products with Varying Wastewater Treatment Processes and Operating Conditions e Conception of a Database and First Results, pp. 49e56.
[37] Bester, K., 2004. Retention characteristics and balance assessment for two polycyclic musk fragrances (HHCB and AHTN) in a typical German sewage treatment plant. Chemosphere 57, 863e870.
[38] Chen, X., Pauly, U., Rehfus, S., & Bester, K. (2009). Removal of personal care compounds from sewage sludge in reed bed container (lysimeter) studies - Effects of macrophytes. *Science of the Total Environment*, *407*(21), 5743–5749. https://doi.org/10.1016/j.scitotenv.2009.07.023

AHTN. These are corresponding also to one other study[39]. After 12 months, the HHCB concentrations were reduced by 25 % in the reed canary grass planted containers, 27 % in the bulrush planted containers, 22 % in the reed planted containers and 23 % in the unplanted containers. Similar to HHCB, AHTN was reduced by 24 % in the reed canary grass planted containers as well as in the bulrush planted containers, 20 % in the reed planted containers and 21 % in the unplanted containers after 12 months.

The highest amount 1.600 ng g^{-1} (dry mass) was determined in the beginning of the project. After thirteen months the OTNE concentrations were reduced by 70 %, 73 %, 72 % and 73 % when using reed canary grass, bulrush, reed and unplanted containers, respectively. The concentrations of Triclosan in the beginning of the experiment was 800 ng g^{-1} (dry mass). After thirteen months the Triclosan concentrations were reduced to less than 50 % and the concentrations were 360, 310, 390 and 360 ng g^{-1} (dry mass) in the reed canary grass, bulrush, reed and unplanted containers. Considering a standard deviation of 12 % from the method validation this difference compared to the starting concentration is significant.

So the sludge reed bed container study showed that the reed bed sludge treatment technology reduces persistent organic pollutants (such as HHCB, AHTN, Triclosan, and OTNE) significantly. After a twelve-month experiment, only 73–78 % of HHCB, 76–80 % of AHTN, 38–48 % of Triclosan, and less than 30 % of OTNE were left in the containers. The decrease of pollutants during the full lifetime (10 years) of reed beds could be much higher[40].

Study[41] showed that the concentrations of musk fragrances decreased with depth in the vertical profile (ambrettolide, 98 %; cashmeran, 93 %; galaxolide, 90 %). An exception was the concentration of tonalide which increased by a factor of two with depth.

1.2.2 Hazardous components

Wastewater and the sludge produced from the wastewater treatment process contain a vast amount of organic micro-pollutants, some of which are undesirable in the environment. Hazardous organic compounds such as linear alkylbenzene sulfonates (LASs) and nonylphenolethoxylates (NPEs) have been reported to be degraded in an STRB with a mineralization of 98 % of LAS and 93 % of NPE[42]. In general, the content of hazardous organic compounds in the sludge residue is reduced to such a degree that the sludge conforms to the limits and norms for deposition on agricultural land[43,44].

Hazardous components include many different groups of organic compounds, among them AOX, absorbable organic halogen; DEHP, di(2-ethylhexyl)phthalate; LASs, linear alkylbenzene lphonates; NP/NPEs, nonylphenols and nonylphenol ethoxylates; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzo-*p*-furans.)[45].

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[39] Mueller J, Boehmer W, Litz NT. Occurrence of polycyclic musks in sewage sludge and their behaviour in soils and plants part 1. J Soils Sediments 2006; 6:231—5.

[40] Chen, X., Pauly, U., Rehfus, S., & Bester, K. (2009). Removal of personal care compounds from sewage sludge in reed bed container (lysimeter) studies - Effects of macrophytes. *Science of the Total Environment*, *407*(21), 5743–5749. https://doi.org/10.1016/j.scitotenv.2009.07.023

[41] Matamoros, V., Nguyen, L. X., Arias, C. A., Nielsen, S., Laugen, M. M., & Brix, H. (2012). Musk fragrances, DEHP and heavy metals in a 20 years old sludge treatment reed bed system. Water Research, 46(12), 3889–3896. https://doi.org/10.1016/j.watres.2012.04.027

[42] Nielsen, S. Mineralisation of hazardous organic compounds in a sludge reed bed and sludge storage. Water Sci. Technol. 2005, 51, 109–117.

[43] Nielsen, S. Helsinge sludge reed bed system: Reduction of pathogenic microorganisms. Water Sci. Technol. 2007, 56, 175–182.

[44] Nielsen, S.; Bruun, E.W. Sludge quality after 10–20 years of treatment in reed bed systems. Environ. Sci. Pollut. Res. 2015, 22, 12885–12891.

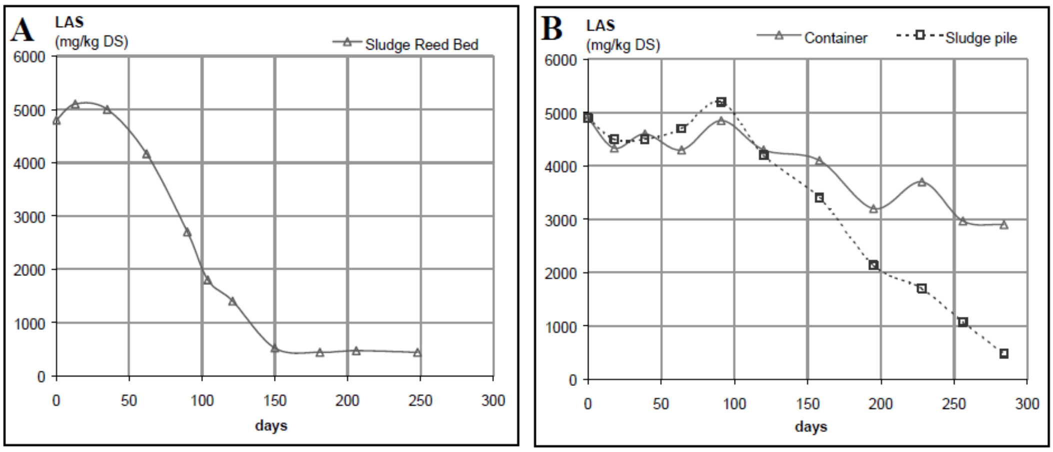
[45] Smith, S. R. (2009). Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *367*(1904), 4005–4041. https://doi.org/10.1098/rsta.2009.0154

Experts from reviewed literature are most often concerned with hazardous organic compounds (PAH, DEHP, LAS, NPE) and their biodegradation products and mineralization[46].

Organic chemicals discharged in urban wastewater from industrial and domestic sources, or those entering through atmospheric deposition onto paved areas via surface run-off, are predominantly lipophilic in nature and therefore become concentrated in sewage sludge, with potential implications for the agricultural use of sludge as a soil improver. Biodegradation occurs to varying degrees during wastewater and sludge treatment processes. However, residues will probably still be present in the resulting sludge and can vary from trace values of several micrograms per kilogram up to approximately 1 % in the dry solids for certain bulk chemicals, such as linear alkylbenzene sulphonate, which is widely used as a surfactant in detergent formulations. However, the review of the scientific literature on the potential environmental and health impacts of organic contaminants (OCs) in sludge indicates that the presence of a compound in sludge, or of seemingly large amounts of certain compounds used in bulk volumes domestically and by industry, does not necessarily constitute a hazard when the material is recycled to farmland. Furthermore, the chemical quality of sludge is continually improving, and concentrations of potentially harmful and persistent organic compounds have declined to background values. Thus, recycling sewage sludge on farmland is not constrained by concentrations of OCs found in contemporary sewage sludges[47].

Mineralisation of LAS and NPE in a sludge reed bed

In study »Sludge treatment and drying reed bed systems Nilsen et al states concentrations of LAS and NPE in the sludge. The concentrations of LAS in samples was approximately 5.000 $mgkg^{-1}$ dry matter (Graph 2). During the period from day 35 (30. 03. 1999) to day 150 (23. 07. 1999), the concentration of LAS was reduced by 90 %. At the end of the experiment, the sludge had a LAS content of approximately 100 $mgkg^{-1}$ dry matter, which represents approximately 2% of the initial concentration (**Error! Reference source not found.**). The total mass of LAS added at the start of the experiment was approximately 24,5 kg. After 9 months there was 0,4 kg remaining.



Graph 2: Mineralisation of LAS in the digested sewage sludge. Concentration (mg/kg dry matter) as a function of time. A: sludge reed bed. B: container and sludge pile[48].

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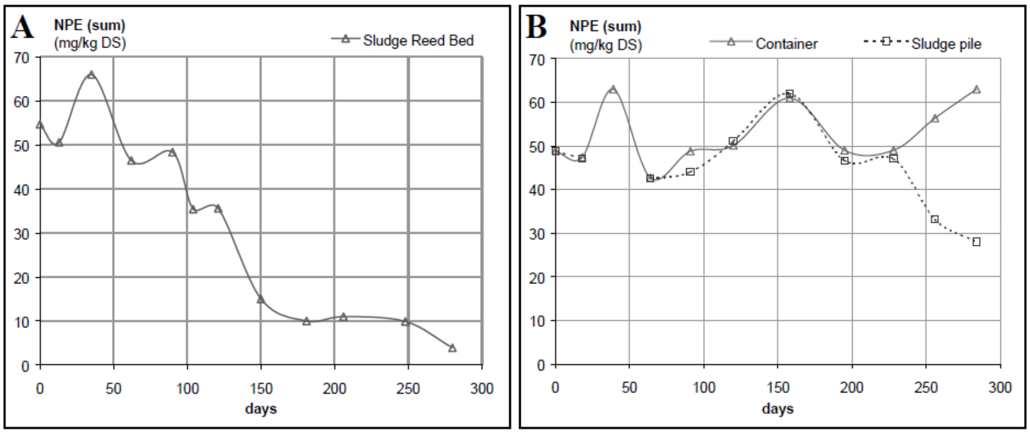
[46] Gustavsson, L., & Engwall, M. (2012). Treatment of sludge containing nitro-aromatic compounds in reed-bed mesocosms - Water, BOD, carbon and nutrient removal. *Waste Management*, *32*(1), 104–109. https://doi.org/10.1016/j.wasman.2011.08.016

[47] Smith, S. R. (2009). Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *367*(1904), 4005–4041. https://doi.org/10.1098/rsta.2009.0154

[48] Danish Environmental Protection Agency (DEPA). 2001. Spildevandsslam fra kommunale renseanlæg i 1999. Orientering fra Miljøstyrelsen (waste-water sludge from municipal waste-water treatment plants in 1999 and . Orientation from the Danish EPA) No. 3, (In Danish)

The concentration of NPE (total) at the start of the experiment (23.02.1999) was approximately 54 mg kg^{-1} dry matter (Graph 3). The total mass of NPE added at the start of the experiment was approximately 0.3 kg. After nine months, there was 0.02 kg remaining. During the course of the experiment (284 days), the concentration of NPE (total) in the sludge residue was reduced by a total of approximately 93 % to a total concentration of approximately 4 mg kg^{-1} dry matter at the end of the experiment. Mineralisation of NPE in the sludge pile resulted in a 43% reduction (Graph 3)[49].



Graph 3: Mineralisation of NPE in digested sewage sludge. Concentration ($mgkg^{-1}$ dry matter) as a function of time with treatment in A: sludge reed bed. B: container and sludge pile[50].

Concerning toxic organic compounds (Table 3), the study[51] shows a dramatic decrease in their concentrations in the sewage sludge. However, several fluctuations were observed during the time, probably due to the high variability in sludge loading concentrations. Nevertheless, the effectiveness of RBs in degrading toxic organic compounds was clearly proven by the concentrations (2.600 $mgkg^{-1}$ dw for LAS; 100 mg/kg dw for DEHP; 50 $mgkg^{-1}$ NPE) obtained for all the target compounds, which were below the guideline limits stated in the European Union's Working Document on Sludge (2000), for land application. Moreover, the trend of the contamination index (CI), which expresses the level of contamination normalized to total organic matter (TOC), reached very significant low values (at 42 months, 66 months and 78 months), thus confirming that there were synergic actions in roots and microorganisms within beds established. This synergic action is mainly due to oxygen diffusion in sludge: reeds enable oxygen to diffuse from the atmosphere to their root system, which boosts the population and activity of naturally occurring aerobic microorganisms, which, in turn, mineralize the sludge[52,53]. Other studies have shown a decrease in the levels of toxic organic compounds in reed beds stressing the need for aerobic conditions to enhance their degradation[54,55,56].

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[56] Matamoros V, Nguyen LX, Arias CA, Nielsen S, Laugen MM, Brix H (2012) Musk fragrances, DEHP and heavy metals in a 20 years old sludge treatment reed bed system.Water Res 46:3889–3896. doi:10. 1016/j.watres.2012.04.027

Table 3: Di-2-ethylhexyl-phthalate (DEHP, mg/kg dw); Linear alkyl benzene sulfonate (LAS, mg/kg dw); nonylphenol and nonylphenol ethoxylates with 1 or 2 ethoxy groups (NPE, mg/kg dw)[57]

MONTHS	DEHP	LAS	NPE	CI
6	6,22 b	51 d	41 d	3,6 e
18	5,7 b	46cd	32 d	2,7 d
30	3,4 ab	40 c	20, c	1,8 c
42	< 1,00 a	3,00 a	25 c	0,9b
54	5,3 b	32 c	25 c	2,0 c
66	4,1 b	8,5b	4,5 b	0,6 ab
78	< 1,00 a	< 3,00 a	< 3,00 a	0,3 a

DEHP behaved differently to all other compounds studied, as the concentration of DEHP decreased in the upper 40 cm, increased in the middle depths, and decreased again in the bottom of the profile (overall attenuation ca. 41 %). In a study from 2009[58] a removal from 40 to 70 % in a RBs after 13 months of incubation is reported, whereas Jensen and Jepsen[59] observed that the abundance of DEHP in Danish sludge was reduced nearly to half from 1995 to 2002 to comply with Danish standards. This indicates that the variability in the concentration of DEHP in the vertical sludge profile may be affected by the different use of DEHP in the past, combined with removal processes in the sludge residue. The lack of a significant decreasing concentration with sludge age indicates that DEHP is very persistent in RBs. However, a Danish monitoring program showed a 60 % reduction of DEHP in digested sludge treated in RBs in the top layer of the sludge residue[60].

Experience has shown that the quality of the final product with respect to mineralization of hazardous organic compounds after treatment enables the application of the biosolids to agriculture as an enhanced treated product[61,62].

1.2.3 Antibiotics

Antibiotics contamination and related antibiotics resistance genes (ARGs) in wastewater sludge have been a concern for environmental pollution and human health risk for years. Study[63] investigated the fate of antibiotics and related ARGs in biosolids from reed beds. In the study, three sludge treatment reed beds of different technology were examined. No.1 was a sludge drying bed with aeration tubes; unit No.2 was a ventilated sludge drying reed bed; and the unit No.3 was a sludge drying reed bed without aeration tubes. The targeted antibiotics included oxytetracycline, roxithromycin, and azithromycin. The targeted ARGs included tetA, tetC, msrSA, and ermB. The results indicated that in all three units, antibiotics were removed significantly, and related ARGs declined over one-year period. The antibiotics concentrations in the surface layer were lower than those in the bottom layer. The highest removal efficiency of the targeted antibiotics was observed in the unit No.2.

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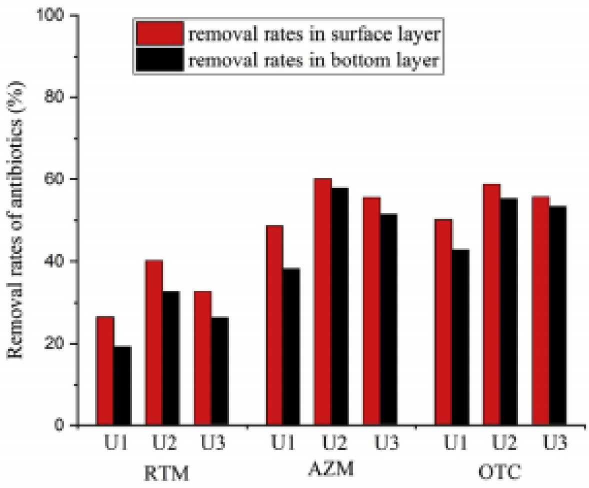
[63] Ma, J., Cui, Y., Zhang, W., Wang, C., & Li, A. (2019). Fate of antibiotics and the related antibiotic resistance genes during sludge stabilization in sludge treatment wetlands. Chemosphere, 224, 502–508. https://doi.org/10.1016/j.chemosphere.2019.02.168

During a period for about 12 months of prolonged sludge stabilization, the concentrations of the three antibiotics declined in all three units while the antibiotics residual concentrations in the bottom layer were higher than those in the surface layer. Obvious fluctuation of RTM, AZM and OTC concentrations were detected in different layers in all three units, dropped on an average from 23 $mgkg^{-1}$ dw to 15,5 $mgkg^{-1}$ dw in the surface layer and 26 $mgkg^{-1}$ dw to 18,9 mg/kg dw in the bottom layer, from 709 $mgkg^{-1}$ dw to 324$mgkg^{-1}$ dw and 895 mg/kg^{-1} dw to 451 mg kg^{-1} dw, from 929 mgkg dw to 365 mgkg dw and 929 $mgkg^{-1}$ dw to 551 $mgkg^{-1}$ dw, respectively. The removal efficiency of the three antibiotics in the surface layer were higher than that of the bottom layer, with the second unit had the highest removal efficiency. Comparing the second unit with the others, the reeds and aeration tubes enabled efficient air exchange, creating partial aerobic conditions that allowed the aerobic microorganisms to rapidly multiply which removed antibiotics from the sludge effectively[64].



Graph 4: The removal efficiency of RTM, AZM and OTC.

U1 represented the sludge samples from the first sludge treatment wetland, which was the traditional sludge drying bed. U2 represented the sludge samples from the second sludge treatment wetland, which was the sludge drying reed bed with aeration tubes. U3 represented the sludge samples from the third sludge treatment wetland, which was sludge drying reed bed without aeration tubes[65] The graph suggests that there is higher removal when passive aeration pipes are installed therefore the sludge drying reed bed with ventilation structure has the best effect on the removal of antibiotics.[66]

Another study[67] showed that after three years, approximately 41–72 % of CIP and 49–84 % of AZM were eliminated from the influent.

1.3 Nutrients

Sewage sludge provides essential nutrients for plant growth. Biosolids can supply N, P, sulphur and other nutrients to soils. The concentration of nutrients in biosolids depends on sewage composition and treatment, and on subsequent sludge management. In terms of nutrients, we are interested mainly in N, P concentrations.

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Nutrient concentrations are not regulated unless the sludge is to be sold as organic fertilizer following; for example, a composting treatment (Regulation (CE) No. 2003/2003). However, the concentration of nutrients is needed to ensure appropriate dosages of the sludge. For instance, phosphorus doses required for agriculture application are strictly dependent on the fertilizer and soil characteristics[68].

Concerning the nutrients, a relatively high concentration of nitrogen (2,4-5,0 % TS) is quite common in secondary sludge. However, the mineral and organic nitrogen chemical forms are strictly dependent on the wastewater origin and treatment. Thus, considering fertilizing properties, only the nitrogen mineral fraction should be taken into account, due to its readily available form for crops. Thus, considering fertilising properties, only the nitrogen mineral fraction should be taken into account, due to its readily availability form for crops. Other macronutrients, such as phosphorus and potassium, are usually found in mineral form. The major proportion of phosphorus expressed as P_2O_5 ranges from 0,8-2,8 % TS in primary sludge to 0,5 to 0,7 % TS in secondary sludge[69].

The average concentration of nitrogen in the stabilized sludge varied from 1,9 ± 0,2 to 2,4 ± 0,3 % of dry matter. The average concentrations of nitrogen in dewatered sewage sludge varied from 1,5 to 5,0 % dry matter[70]. A decrease of Kjeldahl nitrogen concentration in stabilized sludge profile was observed. This was also confirmed by other authors[71,72].

The average concentrations of phosphorus compounds in dewatered sewage sludge vary from 0 to 1,5 % dry matter. The average values of phosphorus concentrations determined in analyzed sludge were from 3,8 to 4,7 % dry matter. Such a high concentration of phosphorus could be caused by the high consumption of detergents[73].

A Study[74] detected a certain decrease in nutrient concentration (TN and TP) along the vertical profile of sludge treated in RBs, probably due to plant uptake during the growing season. The same pattern was detected by Pempkowiak and Obarsza-Pempkowiak[75] in sludge systems in Poland.

Although they are essential for plant growth, nutrients (mainly N and P) can be harmful when excessively applied. Different studies have shown both N,[76,77] and P accumulation in sludge-amended soils[78]. It is well known that over-application of N can lead to nitrate contamination of groundwater, although such a risk is reduced if nutrients are applied at agronomic rates[79].

The results confirm that the long-term stabilization of sewage sludge causes an increase of phosphorus concentration, which is caused by the decomposition of organic matter while retaining the

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phosphorus. The presence of nutrients N and P indicates that the stabilized sludge in the RBs can be a valuable fertilizer80. The results of nutrients suggest that the final product from the treatment, because of its stabilization, may be used as a fertilizer in agriculture.

One study[81] reported that reed beds have over 90 % removal efficiency for sludge dewatering, total suspended solids, and biochemical oxygen demand, and that nitrates and total phosphorus removal rates were 90 and 80% of the initial concentrations, respectively. Analysis of the biosolids showed the content of N $mgkg^{-1}$ 170 and P $mgkg^{-1}$ 360[82].

1.4 Total Solids and Total Volatile Solids

Sewage sludge is the leading organic waste or by-product generated in wastewater treatment plants (WWTP). In general, it has a total solids concentration around 1–3 %, depending on the treatment process[83].

The efficiency of several RBs for sludge dewatering, as shown by the increase in totals solids (TS) concentration in the sludge, can be observed in Table 4. In general, TS concentration increases from 1–4 % in influent sludge to 20–30 % within the reed beds. Even higher TS values were reported from Polish systems (58 %), although they might result in a higher TS concentration in feeding sludge (4–10 %) and the fact that it was a primary sludge.

Table 4: Total solids (TS) and Volatile solids (VS) concentration observed in several RBs.

SYSTEMS' LOCATION	SOURCE OF THE SLUDGE	TS (%)		VS (%TS)		REFERENCE
		Influent	RBs	Influent	RBs	
Fort Campbell, USA	Anaerobic digestion	3	32*	-	46*	Kim and Smith (1997)
Pilot plant in Rugeley, Staffordshire, UK	Biological Areated Filter (BAF) and raw slurry solids	4	20	74	52	Edwards et al. (2001)
Darzlubie, Poland	Imhoff tank	4-10	58	~60	45	Obarska-Pepkowiak et al. (2003)
Helsinge, Denmark	Activated sludge and activated slodge from settling tank	0,5-0,7	20	-	-	Nielsen (2003)
Alpens, Spain	Activated sludge, extended aeration	0,7-1,5	22-25*	52-67	39-42*	Uggetti et al. (2009a)
St Boi de Lluçanès	Activated sludge, extended aeration	3	20-28*	52-42	36-40*	Uggetti et al. (2009a)
Seva, Spain	Activated sludge, contact-stabilization	0,3-2	15-20*	58-59	46-50*	Uggetti et al. (2009a)

*Average from different depths

[80] Kominko, H., Gorazda, K., & Wzorek, Z. (2019). Potentiality of sewage sludge-based organo-mineral fertilizer production in Poland considering nutrient value, heavy metal content and phytotoxicity for rapeseed crops. *Journal of Environmental Management*, *248*(February), 109283. https://doi.org/10.1016/j.jenvman.2019.109283

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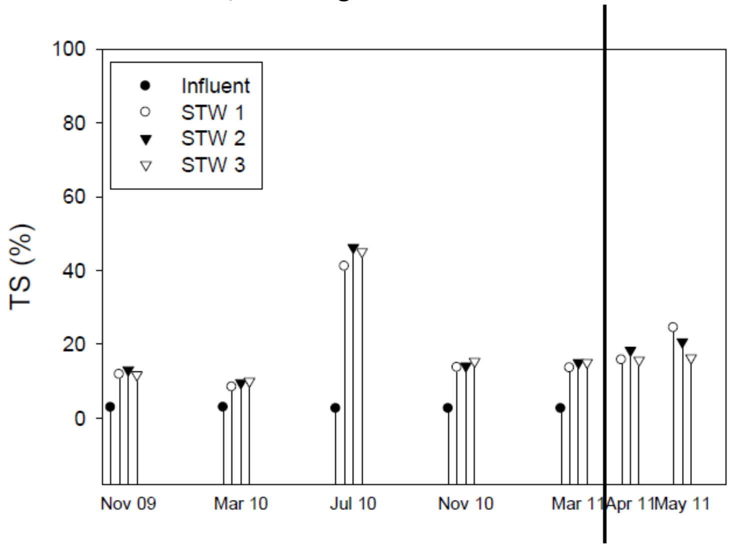
An analysis of Kucicino from June 2019 treated (dried) sludge showed that biosolids had a 24 % TVS content indicating high treatment performance, high mineralization rates, and proper treatment of sludge drying reed beds in Kucicino. The TVS content dropped from approximately 90 % TVS (in fresh sludge) to 24 % of treated sludge. The requested value of 66 % TVS in treated sludge has therefore been far exceeded. TS levels were always under the required values (with one exception)[84].

During sludge treatment within the reed beds, a VS reduction of 25–30 % can be achieved, reaching final VS concentrations of between 40 and 50 %. VS removal yields depend on influent sludge VS concentration. For instance, sludge from extended aeration activated sludge systems has lower VS content than that from other treatments (i.e., conventional activated sludge); hence VS removal within the wetlands is lower when this type of sludge is treated. Consequently, the efficiency in terms of VS removal of the wetlands might be slightly lower than that of aerobic digestion (40–55 %) or anaerobic digestion (35–50 %)[85,86]. On the other hand, VS contents in compost are considerably higher (60–70 %) than in sludge from other treatments, including wetlands.[87]



Graph 5: Total solids concentration measured within the influent sludge and the three wetlands during the feeding and resting period. The vertical line corresponds to the last sludge loading[88].

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[84] SWWTP-KARBINCI-FINAL REPORT OF PROVIDED ACTIVITIES AND SERVICES DURING DEFECT LIABILITY PERIOD FROM 25. 09. 2017. UP TO 24. 09. 2019.

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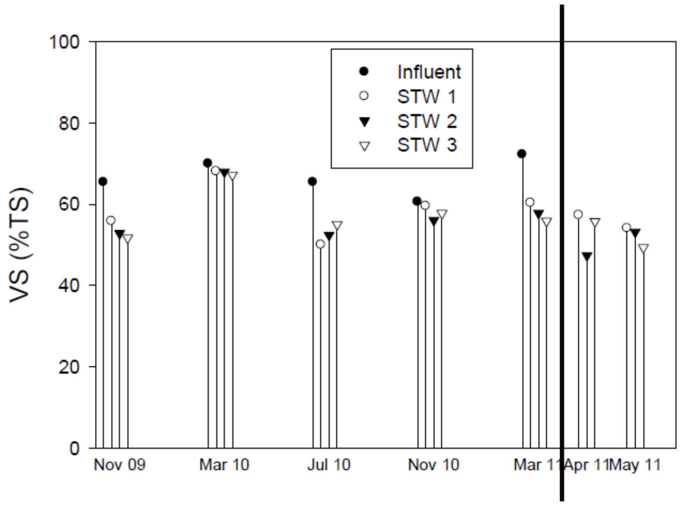
[87] Uggetti, E., Ferrer, I., Llorens, E., & García, J. (2010). Sludge treatment wetlands: A review on the state of the art. *Bioresource Technology*, *101*(9), 2905–2912. https://doi.org/10.1016/j.biortech.2009.11.102

[88] Uggetti, E., Ferrer, I., Llorens, E., & García, J. (2010). Sludge treatment wetlands: A review on the state of the art. *Bioresource Technology*, *101*(9), 2905–2912. https://doi.org/10.1016/j.biortech.2009.11.102



Graph 6: Volatile solids concentration measured within the influent sludge and the four wetlands during the feeding and resting period. The vertical line corresponds to the last sludge loading[89].

1.5 Pathogens

Microorganisms transmitted by the fecal–oral route are usually referred to as enteric pathogens because they infect the gastrointestinal tract. Pathogens are characteristically stable in water and food and, in the case of enteric bacteria, are capable of growth outside the host under the right environmental conditions (warm temperatures and sufficient organic matter). Waterborne diseases are transmitted through the ingestion of contaminated water, which serves as the passive carrier of the infectious agent. The classic waterborne diseases, cholera and typhoid fever, which frequently ravaged densely populated and with poor hygiene areas throughout human history, have been effectively controlled by the protection of water sources and by treatment of contaminated water supplies. Other diseases caused by bacteria, viruses, protozoa, and parasitic worms (helminthes) may also be transmitted by contaminated drinking water. However, it is important to remember that waterborne diseases are transmitted by the fecal–oral route, from human to human or animal to human, so that drinking water is only one of several possible sources of transmission. Disease-causing microorganisms are almost always present at some level in domestic sewage[90]. That is why the disposal of biosolids is still so undesirable.

Wastewater sludge contains a large number of bacteria. Salmonella, Coli bacteria and faecal Streptococci are found in wastewater sludge (raw and mesophillic-digested). As a general rule, pathogenic bacteria which are excreted and end in an alien environment, only live for a short period of time depending upon various environmental factors and the bacteria's own characteristics[91].

The reduction of pathogenic bacteria in reed beds was observed[92,]. Salmonella, enterococci and Escherichia coli was investigated using the sludge reed bed system. The system at Helsinge was established in 1996 and had a capacity of 630 tonnes of dry solids per year and consists of 10 basins. The total sludge residue height in April 2006 was approximately 1.40 m. The sludge (about 0.5–0.8% dry solids), with which the individual basins were loaded, contained a large number of bacteria. Salmonella, enterococci and E. coli were found in the sludge in the following quantities: 10–300/100 g

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(wet weight), 7,000–25,000 CFUg^{-1} (wet weight) and 800,000–10,000,000 CFUg^{-1} (wet weight), respectively.

Even after 1–3 days following the end of sludge feeding, the reduction in pathogenic organisms was found to be significant. Particularly in the case of Salmonella the number was fewer than two microorganisms per 100 g of sludge after 2–7 days. For enterococci and E. coli, the reduction was approximately 5, 6–7 log units in a period of 2–3 months, respectively. These results suggest that only the top layers (0–0.10m and 0.10–0.25 m) of the sludge residue will be recontaminated during the loading. The sludge residue below 0.25m does not appear to be recontaminated during every new loading[93].

Analysis of the reduction in pathogens in the sludge residue from Galten sludge reed bed plant[94] in sludge residue samples taken 6 - 9 months after the last loading indicated that the pathogen content was reduced by approximately 6 log units based on a dry solids basis to a level corresponding to the requirements for controlled sanitation. The results are in agreement with results reported by the Danish EPA for the storage of sludge (Environmental project number 351 regarding sanitation aspects during handing and recycling of organic waste).

Table 5: Concentration of pathogens in sludge residue after treatment in sludge reed beds for 6-9 months[95]

Salmonella	Not detected
Faecal Streptococci	Less than 100/g
E. Coli	Less than 20/g

Experience has shown that the quality of the final product with respect to heavy metals, hazardous organic compounds and pathogen removal after 10 years of treatment make it possible to recycle and reclaim the biosolids for agriculture as an Advanced Treated product[96,97,98,99,100,101].

[93] Cooper, D. J., Orbicon, A. S., & Roskilde, D.-. (n.d.). SLUDGE TREATMENT IN REED BEDS SYSTEMS – DEVELOPMENT , EXPERIENCE – TREATMENT OF WATER WORKS SLUDGE AND SAS - CASES Keywords Loading – Operational Strategy. (Table 1).
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