

Sewage sludge hazard index based on bioassays: strategic tool for the decision-making process on sludge agricultural use

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ABSTRACT

A Sewage Sludge Hazard Index (SSHI) based on short term and low cost bioassays was developed as a complementary tool for the decision-making process involving sewage sludge application to agricultural land. SSHI integrates results from *Vibrio fischeri*, *Daphnia similis* and seed elongation/germination test. The proposed index is calculated as the natural logarithm of one plus the number of positive toxic responses multiplied by the average of toxic units obtained for each bioassay. It was calculated for 28 samples from 7 different wastewater treatment plants (WWTP) of Sao Paulo State and ranged from 0.3 to 4.8. The frequency of samples non-compliances was calculated for index-rank considering the pollutants thresholds for sewage sludge derived from different norms. SSHI below 2 seems to warrant compliance with Brazilian, US and EU legal values and it seems to be a promising tool for assessing hazard degree of sewage sludge. Additional chemical and toxicological data from different WWTP samples should be considered for a better validation of this index.

Keywords: hazard index, sewage sludge, phytotoxicity, acute toxicity, metals; organic pollutants;

RESUMO

Um Índice de Perigo de Lodo de Esgoto (IPLE) baseado em testes ecotoxicológicos de baixo custo e rápida duração foi desenvolvido como ferramenta complementar para auxiliar o processo de decisão sobre o aproveitamento em solo agrícola. IPLE integra resultados de testes com *Vibrio fischeri*, *Daphnia similis* e alongamento e germinação de sementes. O índice proposto foi calculado como logaritmo neperiano de 1 somado à multiplicação do número de testes com resultado positivo pela toxicidade média. O índice foi calculado para 28 amostras de 7 diferentes Estações de Tratamento de Efluentes (ETE) do estado de São Paulo e tiveram variação entre 0,3 e 4,8. Foi verificado a frequência de amostras por faixa de resultado do índice quanto à conformidade com as regulamentações do Brasil, Estados Unidos e Europa. O IPLE abaixo de 2 mostrou atender aos requisitos das normas nacionais e internacionais e mostrou ser uma ferramenta promissora para avaliação da periculosidade de poluentes no lodo de esgoto. Um número maior de amostras incluindo outras ETEs devem ser consideradas para aprimoramento da validação do índice.

1 - INTRODUÇÃO

Sewage sludge is a highly complex waste that results from treatment processes in Wastewater Treatment Plants (WWTP) (Singh and Agrawal, 2008). The disposal of sewage sludge has become a worldwide environmental problem. In developed countries like US and the European Union ten millions tonnes of sewage sludge are produced per year (Carbonell et al., 2009; McClellan and Halden, 2010).

Sewage sludge characteristics vary with the treated wastewater quality, sewage/sludge treatment processes and sludge storage (Parnaudeau et al., 2004). Toxic metal in urban and industrial waste water can be present in levels ranging from 0.5 to 2 % of the sewage sludge dry weight, reaching 6% in extreme conditions (Renoux et al., 2001). For the last 50 years synthetic organic chemicals production for industrial and urban use has increased dramatically (Rogers, 1996), as a consequence, the diversity and concentration of contaminants in waste water and in sewage sludge is expected to increase accordingly.

The main disposal route of sewage sludge has been sanitary landfill. Since the eighties, agricultural use has become an option (USEPA, 1993), because this material can be applied to the soil providing nutrients and organic matter, improving soil quality. Several countries have issued regulatory standards for this activity. In the United States (US) and the European Union (EU), for a sewage sludge to be accepted for agricultural use representative samples must be analyzed for heavy metals, pathogens, agronomic characteristics and stability (Council, 1991; USEPA, 1993). More recently, Brazil has regulated this practice (Brasil, 2006a) based on standards developed by the US Environmental Protection Agency (EPA) (USEPA, 1993).

Regulations provide threshold acceptance values for sludge agricultural use based only on total concentrations of a limited number of inorganic compounds. This approach covers only part of the knowledge necessary to evaluate and assess the toxic potential of sewage sludge for humans and the ecosystem (Alvarenga et al, 2007; Mantis et al., 2005; Schnaak et al, 1997) and does not consider the bioavailability of metals and organic compounds (Alvarenga et al, 2007; Peralta-Videa et al., 2009) or their additive or synergic interactions (Chen and Lu, 2002). So that the use of sewage sludge on agricultural land can be an important pathway for human exposure to persistent pollutants (Hale et al. 2001), and raises concerns regarding food

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safety and long-term soil productivity (Renoux et al., 2006; Singh and Agrawal, 2008).

Municipal WWTP, especially in Brazil, treats together urban and industrial effluents in different proportions and pluvial waters carrying contaminants from aerial deposition and run-off. For many persistent hydrophobic organic chemicals, adsorption to the sewage sludge solids is the primary pathway for their removal from waste water (Clarke and Smith, 2011; Harrison et al., 2006).

The regulation of organic compounds in sewage sludge has presented a challenge for environmental agencies. USEPA performed a five year study to estimate the national levels of PCDD/F in sewage sludge and only 61 of the 6,857 samples exceeded the 300 ppt threshold value (USEPA, 2002). The low incidence of relatively high levels of PCDD/F was considered an acceptable risk to human health and so it was decided not to regulate these compounds in biosolids (USEPA, 2003). USEPA verified the occurrence of more than 800 chemicals in sewage sludge, but only 40 with sufficient data to allow the Agency to either conduct exposure and hazard assessments or determine if a regulatory action may be required (USEPA, 2007). More recently a targeted national sewage sludge survey was performed to complement information about other pollutants in sewage sludge, including 145 different chemicals and the need for further actions is still being evaluated (USEPA, 2009).

Directive 86/278/EEC does not include specific limits for organic contaminants. Some European Member States have set limits for organic compound groups, while others have not (RPA, 2010). Since 2000 the European Community is discussing the inclusion of threshold values for organics in biosolids such as halogenated organic compounds (AOX), linear alkylbenzene sulphonates (LAS), di(2-ethylhexyl)phthalate (DEHP), nonylphenol and nonylphenoethoxylates (NPE), polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), polychlorinated dibenzodioxins/ dibenzofuranes (PCDD/F) (EC, 2000). This inclusion will imply in higher costs for the sludge characterization and would derail the disposal in agricultural land for several WWTP.

Some studies suggest that the integration of chemical and ecotoxicological analyses is necessary for a comprehensive hazard characterization of sewage sludge (Alvarenga et al., 2007; Farre and Barcelo, 2003; Mantis et al., 2005). Hazard indexes have been developed for different environmental

matrices by several organizations in order to integrate evidences in a simple effect-based hazard assessment aimed to facilitate the decision-making process regarding environmental and human protection. Water Quality Index is used in several countries to alert, specially non-expert, about water quality of distinct water bodies, and also to signal the necessity for further action (Brown et al 1970). In 1993, Environment Canada developed the Potential Ecotoxic Effects Probe (PEEP), a scientifically management tool, based on ecotoxicological principles, simple to use and interpret, with a good discriminatory potential to assess wastewater toxic loads. It was applied to prioritize corrective or preventive actions regarding point source emissions in Saint-Lawrence River Canada (Blaise and Férard, 2005; Costan et al, 1993).

Those indexes are composed by physico-chemical and toxicological variables. Bioassays have proved to be a good complementary tool to provide better information and reduce uncertainties regarding its hazard (Chapman, 2007). Therefore the development of a sewage sludge hazard index, especially if based on low cost and simple bioassays, could be an interesting tool to help decision making process regarding the safe disposal of sewage sludge. Brazilian regulation recognized the need of a bioassay approach to complement the evaluation of sludge samples when agricultural use is intended (Brasil, 2006a).

The aim of this study was (i) to develop a Sewage Sludge Hazard Index (SSHI) based on short-term and low-cost bioassays, to be used as a complementary screening tool in the early rejection of sewage sludge as soil amendment in agricultural land, (ii) to test the Index with data generated by the Environmental Protection Agency of São Paulo State (CETESB), (iii) and to verify its applicability comparing index levels and non compliance sample frequency with chemical threshold values from different legal norms.

2 - MATERIAL AND METHODS

2.1. Sample collection, processing and analysis

Treated representative sludge samples from seven different municipal wastewater treatment plants (WWTP) of Sao Paulo State Brazil (Table 1) were collected (8 Kg) by CETESB in each season, from April 2007 to January 2008. A total of twenty eight sludge samples were properly storage and carried to the laboratory under 4 °C controlled temperature.

Table 1 – Main characteristics of the wastewater treatment plants studied

WWTP	Treatment Process		Wastewater Source
	Liquid phase	Solid phase	
AT-1	Conventional activated sludge	Digestion, filter press	Urban and industrial effluent from São Paulo metropolitan region
AT-2	Conventional activated sludge	Digestion, filter press	Urban and industrial wastewaters from chemical industries including dye factories
AT-3	Extended aeration with activated sludge	Filter press	Urban effluent from São Paulo city
PCJ-1	Biological filter	Digestion, centrifugation and drying bed	Urban effluents from Americana city and textile industrial wastewaters
PCJ-2	Aerated Lagoons followed by sedimentary lagoons	Centrifugation and drying bed	Urban effluents from Jundiai city and wastewater from food/drinking manufacturing and wood processing
PCJ-3	Activated sludge	Filter press	Urban effluents from several small cities
SMG	Conventional activated sludge	Digestion, beltfilter press	Urban effluents from Franca city

Source: CETESB, 2009

The same sludge samples was analysed for metals, organics, and different ecotoxicological endpoints. Chemical analyses were performed in in natura sewage sludge samples by reference laboratories (Table 2). All ecotoxicity tests were performed in aqueous extracts prepared with 100g of the sewage sludge in natura; 400 mL of ultrapure water; stirred at 0,6 G for 24 hours at room temperature and centrifuged for 30 minutes at 5000g (Mathews and Hastings, 1987). Aqueous extracts were stored in a refrigerator for a maximum 7 days period before testing. Tests conducted were *Vibrio fischeri* toxicity test (15') according to ISO 11348-3:2007, *Daphnia similis* acute toxicity assay (48h) according to ABNT-NBR 12713/2004 and seed germination/root elongation with two different plants according to USEPA OPPTS 850.4200. Those data were published in two different CETESB reports [CETESB, 2008; CETESB, 2009).

Table 2 – Methods applied in sewage sludge *in natura* samples for chemical characterization

Analysis	Method	Laboratory
SVOC	U.S. EPA 8270C	Analytical Solution
VOC	U.S. EPA 8260C	Analytical Solution
PCBs	U.S. EPA 8082 / U.S. EPA 1668A	Analytical Solution
PCDD/F	U.S. EPA 8290 / 1613	Analytical Solution
As, Se, Sb	SW 846 EPA 2007 method 3051A / SW 846 EPA 1996 method 3050B APHA AWWA WEF 21 ^a Ed. 2005 method 3113	CETESB
Hg	SW 846 EPA 2007 method 3051A / APHA AWWA WEF 21 ^a Ed. 2005 method 3112	CETESB
Ag, Ca, Cd, Co, Cu, Fe, Mg, Ni, Na, Pb, Zn	SW 846 EPA 2007 method 3051A / SW 846 EPA 1996 method 3050B APHA AWWA WEF 21 ^a Ed. 2005 method 3111B e 3210B	CETESB

Source: CETESB, 2009

3 – RESULTS AND DISCUSSION

The developed Sewage Sludge Hazard Index (SSHI) meets all assumptions and is defined as the natural logarithm of one plus the number of positive toxic responses multiplied by the average of toxic units obtained in each bioassay (Equation 1). Toxicity data were expressed in Toxicity Units (TU) calculated as 100/Effective Concentration (EC50%). The hazard index scale range is 0 to infinite. Zero will be obtained if all tests provide negative response, and the maximum value is defined by the number of positive responses from the total tested and the mean toxicity. The greater the index, more hazardous is the sample tested.

$$SSHI = Ln \left[1 + n \times \left(\frac{\sum_{i=1}^n Ti}{N} \right) \right]$$

SSHI = Sewage Sludge Hazard Index;

n = Number of positive results;

N = Number of bioassays performed

T = Toxic Units

Due to the model neperian curve the index sensitivity is very high for low values, between 0 and 2, as small variations in toxic units axis implies large variations of the index response.

Vibrio fischeri toxicity test has been widely used for screening sediment, soil from contaminated systems, wastewater, and sewage sludge, either alone or in combination with a battery of other tests, also an increasing number of comparative studies demonstrated its utility, sensitivity, rapidity and affordability (Alvarenga et al., 2007; Doherty, 2001). This test can be conducted with pore water, groundwater, aqueous elutriates and leachates, organic solvent extracts, or solid-phase samples (Doherty, 2001), each methodology has its particular limitations. Aqueous extract assesses mainly the effects of soluble chemical, organic extraction can be influenced by the toxicity of solvents, and solid-phase can have interference from scattering of light due to turbidity (Doherty, 2001).

Authors reported significant associations or correlations between *Vibrio fischeri* acute toxicity and contaminant concentrations, as aromatic hydrocarbons, chlorinated hydrocarbons and naphthalenes (Schiewe et al., 1985), total PAHs (Jacobs et al., 1993), total PCBs, trichlorobenzene, lead (Santiago et al., 1993), benzo[a]pyrene, phenanthrene (Demuth et al., 1993), copper, oil and grease (True and Heyward, 1990), 2-,3 and 4- chlorophenol, 2,4-di- and 2,4,6-trichlorophenol (Zona et al., 1999), azo reactive dyes from textile dyeing and finishing mill (Neamtu et al., 2003), zinc (Heinlaan et al., 2008). Those findings were dependent on the extraction and cleanup methods. The extract method applied by CETESB provides a conservative scenario of mobile contaminants in the sample, including polar and non polar compounds due to the organic carbon dissolved in the aqueous samples.

Daphnia toxicity test has been used for environmental monitoring of pollutants around the globe and plays an important role in establishing regulatory criteria by government agencies (e.g., US EPA, Environment Canada, Organization for Economic Cooperation and Development, Environment Agency of Japan, Environmental Agency of Sao Paulo) (Shaw et al., 2008). This bioassay can be performed for acute or chronic exposure, and are standardized for species *D.magna*, *D.pulex*, *Ceriodaphnia dubia* and *D. similis* (CETESB, 2009; Shaw et al., 2008).

Daphnia acute toxicity test (CE50 48h) has been reported as more sensitive to other invertebrates and fish ecotoxicity tests for parathion, copper,

cationic surfactant, cadmium (Mark and Solbé, 1998). Authors also reported significant response associations for copper, parathion, lindane, linear alkylbenzene sulphonates (LAS) (Mark and Solbé, 1998), chromium, cadmium, lead, arsenic, nickel, and zinc (Seco et al., 2003), ammonia (Gerald et al, 1990), cathecol, acetone, phenol (Guerra, 2001).

Seed germination root elongation toxicity test was developed to be a screening acute phytotoxicity assessment of chemical substances and mixtures (OECD, 2003; USEPA, 1996). It has been applied in the register of pesticides, and for phytotoxic evaluation of soils, sediments and organic waste (Adam and Duncan, 2002; Czerniawska- Kusza et al, 2006; Oleszczuk, 2010; Oleszczuk et al., 2011; USEPA, 1996; Valerio et al., 2007). Usually more than one species are tested in each assay. Only the highest toxic unit for phytotoxicity was selected to be integrated in the index, this effort contributes for the index to be restrictive and accounts for only one evidence per trophic level.

Considering those three bioassays, phytotoxicity test has the closest ecological relevance to the purpose of the index, although *Vibrio* and *Daphnia* acute toxicity test has complementary sensibility to priority pollutants that are restricted for sewage sludge agricultural use.

The Sewage Sludge Hazard Index (SSHI) was calculated for each aqueous extract sample using the *V. fisheri*, *D. similis* and the highest phytotoxicity score (Table 3). The use of only highest phytotoxicity data was made for the index to be conservative. For comparative puporse the index must be calculated considering the same parameter in all campaigns. Lack of data were bypassed based on previous study that collected and tested fitotoxicity by the same methodology and laboratory. The variation in the SSHI values within each WWTP could be related to differences in the influents (Villar et al 2006) or wastewater treatment processes and sludge treatment (Rogers, 1996; Singh and Agrawal, 2008). *Vibrio fisheri* toxicity assay was the most sensitive, results ranged from non toxic to 103 TU (table 3). *Daphnia similis* acute toxicity results ranged from <1 to 33 TU (table 3). Phytotoxicity results ranged from non toxic to 11.6.

Table 3 – Sewage Sludge Hazard Index (SSHI) and bioassay responses obtained from four sludges samples collected in seven Wastewater Treatment Plant (WWTP) of Sao Paulo State, Brazil

WWTP	S	V. f. TU	D. s. TU	L. s. e. TU	L. s. g. TU	B. j. e. TU	B. j. g. TU	Positive results	Mean Toxicity	SSHI
AT-1	1	1	2.9	1	2.1	1	nt	3	2	1.9
	2	1	4.5	1	2.1	1	1.1	3	3	2.2
	3	1.3	5.9	1	nt	1	nt	3	3	2.2
	4	1	5.5	1	nt	nr	nr	3	3	2.1
AT-2	1	7.6	1.6			12.3*		3	7	3.1
	2	3.7	2.5			12.3*		3	6	3.0
	3	6.9	1.9			12.3*		3	7	3.1
	4	103.1	2.5			12.3*		3	39	4.8
	p*			4	nt	12.3	nt			
	p*			1	nt	1	nt			
AT-3	1	2.2	3.4	1.5	1.3	1.4	nt	3	2	2.1
	2	1	33.3	2.3	1.5	2.2	3.0	3	12	3.6
	3	2.7	4.2	2.1	nt	1.0	nt	3	3	2.3
	4	5.8	3.6	2.5	nt	2.2	1.3	3	4	2.6
PCJ-1	1	9.3	5.3	6.7	11.6	2.1	2.9	3	9	3.3
	2	1.8	3.9	3.4	5.3	3.2	3.8	3	4	2.5
	3	5.2	33.3	5.4	4.7	5.8	5.7	3	15	3.8
	4	1	1.9	1.0	nt	1.1	nt	3	1	1.6
PCJ-2	1	1	1.9			nt*		2	1	1.1
	2	0	1			nt*		1	0	0.3
	3	1	12.8			nt*		2	5	2.3
	4	0	3.2			nt*		1	1	0.7
	p*			nt	nt	nt	nt			
	p*			nt	nt	nt	nt			
PCJ-3	1	nt	1.7	nt	nt	nt	nt	1	1	0.4
	2	1.5	1	1	nt	1.1	nt	3	1	1.5
	3	0.9	1	1	nt	1.1	nt	3	1	1.4
	4	1	1	1	nt	1.0	nt	3	1	1.4
SMG	1	0	3.4			nt*		1	1	0.8
	2	1	3			nt*		2	1	1.3
	3	1	3.8			nt*		2	2	1.4
	4	1b	3.6			nt*		2	2	1.4
	p*			nt	nt	nt	nt			

Source: CETESB, 2009; CETESB, 2008, p* Previous study CETESB, 2007

S = samples; TU = Toxic Unit; nt – non toxic; nr – non realized; * = Data assumed based on a previous study (CETESB, 2007); b= sample not analysed, then data was assumed for index calculation based on the median of previous campaign.

Chemicals compounds were summarized for each WWTP considered and compared to values reported in other countries, and regulatory limits from Brazil, European Community, and USA sewage sludge norms for agricultural use (table 4). Inorganic contents from Sao Paulo sewage sludge were close to the median reported by Fytili and Zabaniotou (2008), except molybdenum concentration that was higher in São Paulo. Organic compounds from Sao Paulo sewage sludge were two to three orders of magnitude below maximum concentrations reported by Harrison, Oakes et al. (2006). Those finds indicate that samples analysed in this study had low to medium amount of contaminants, therefore a higher ecotoxicity is expected for samples with a higher degree of contamination.

Table 4 – Metal and organic analysis of the sewage sludge samples from the CETESB data base (Cetesb 2009b) in comparison with the range reported in other countries sewage sludges survey and norms

Compounds	Sewage Sludge Norms (mg/kg dry matter)				World Sewage Sludge (mg/kg dry matter)		State of Sao Paulo sewage sludge (min-max mg/kg dry matter)						
	Bra	USb	EECc	WDSd	min – max	median	AT-1 (n=4)	AT-2 (n=4)	AT-3 (n=4)	PCJ-1 (n=4)	PCJ-2 (n=4)	PCJ-3 (n=4)	SMG (n=4)
Arsenic	41				1.1 – 230 ^e	10	<2.00 - 5.34	<2.00 - 12.2	<2.00 - 4.4	<2.00 - 2.24	<2.00 - 4.32	<2.00 - 2.64	<2.00 - 2.58
Barium	1300				-		304 – 446	173 - 258	151 - 228	63.5 - 573	518 - 624	323 - 567	119 - 273
Cadmium	39	39	20	5	1 – 3,400 ^e	10	1.85 - 9.11	1.43 - 4.59	0.7 - 5.58	5.41 - 10.9	4.16 - 11	<0.50 - 2.03	<0.50 - 1.61
Lead	300	300	750	500	13 – 26,000 ^e	500	87.1 – 138	112 - 209	15.9 - 63.8	102 – 143	153 - 222	4.68 - 31.9	21.8 - 67.2
Copper	1500	1500	1000	800	84 – 17,000 ^e	800	715 – 978	300 - 463	405 - 1075	77.9 – 344	203 - 366	196 - 289	140 - 380
Chromium	1000	1200		800	10 – 990,000 ^e	500	566 – 773	242 - 1508	298 - 631	95.2 – 724	261 - 368	22.9 - 52.3	12 - 344
Mercury	17	17	16	5	0.6 – 56 ^e	6	1.23 - 2.64	0.52 - 2.7	0.4 - 0.79	<0.10 - 2.16	<0.10 - 2	0.38 - 0.53	0.14 - 0.57
Molybdenum	50				0.1 – 214 ^e	4	<15.0 - 31.5	101 - 434	<15.0 - 15.9	<15.0 - 2.68	<15.0 - 48.3	<15.0 - 16.8	<15.0
Nickel	420	420	300	200	2 – 5,300 ^e	80	<4.00 – 334	<4.00 - 811	95.6 - 138	51.7 – 115	31.2 - 42.6	8.73 - 25.9	18.6 - 87.1
Selenium	100				1.7 - 17.2 ^e	5	<2.00 - 3.54	<2.00 - 5.11	<2.00	<2.00 - 4.2	<2.00	<2.00 - 2.02	<2.00 - 2.19
Zinc	2800	2800	2500	2000	101 – 49,000 ^e	1700	1397 – 2132	659 - 5923	571 - 4688	1095 - 1525	1244 - 1644	282 - 787	238 - 930
DEHP ¹				100	nd-58,300 ^{f,2}	-	<0.005	<0.005 - 54.7	<0.005 - 128	<0.005 - 15.4	<0.005 - 95.2	<0.005	<0.005
PAH ³				6	nd-199 ^{f,4}	-	9.4E-01 - 2.8E+00	1.9E-01 - 9.1E+00	<0.005 - 1.4E+00	<0.005 - 1.0E+02	<0.005	<0.005	<0.005 - 6.5E-02
other PAH5						-	6.1E-01 - 2.2E+00	5.0E-02 - 2.4E+00	<0.005 - 3.2E-01	1.1E-02 - 1.8E+01	4.5E-02 - 7.2E+00	<0.005	<0.005 - 2.2E-02
PCB ⁶				0.8	nd-765 ^f	-	1.3E-01 - 1.7E+01	5.3E-02 - 3.1E-01	7.3E-03 - 5.7E-02	9.4E-03 - 2.9E-02	1.7E-02 - 1.2E+00	2.5E-03 - 5.8E-03	9.0E-04 - 3.8E-03
other PCB						-	1.1E-01 - 2.3E+01	4.3E-02 - 2.3E-01	1.7E-03 - 7.2E-02	3.7E-03 - 2.3E-02	2.1E-02 - 1.9E+00	1.1E-03 - 8.6E-03	6.0E-04 - 7.5E-03
PCDD/F ⁷				1.0 E-4	1.1E-06-4.1E-03 ^f	-	5.1E-06 - 3.2E-05	3.8E-05 - 9.9E-05	2.0E-06 - 1.1E-05	2.3E-05 - 8.3E-05	6.0E-06 - 6.5E-06	8.2E-08 - 9.2E-07	6.2E-07 - 1.1E-05
Chlorobenzenes ⁸					Nd-184 ^f	-	<0.005 - 1.0E-01	2.2E-01 - 5.9E-01	<0.005 - 3.7E-02	<0.005 - 8.0E-03	<0.005	<0.005	<0.005 - 1.0E-01

Source: Adaptated from CETESB, 2009; (a) Brasil, 2006; (b) Council, 1991; (c) USEPA, 1993; (d) EC, 2000; (e) Fytli and Zabaniotou, 2008; f (Harrison et al, 2006);

(1) Bis(2-ethylhexyl) phthalate; (2) phthalates; (3) Sum of the following polycyclic aromatic hydrocarbons: acenaphthene, phenanthrene, fluorene, flouranthene, pyrene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, indeno(1, 2, 3-c, d)pyrene; (4) polycyclic aromatic hydrocarbon; (5) Sum of the following polycyclic aromatic hydrocarbons: Acenaphthylene, Anthracene, Benzo (a) anthracene, Chrysene, Naphthalene; polychlorinated biphenyl; (6) Sum of the polychlorinated byphenils components number 28, 52, 101, 118, 138, 153, 180; (7) PCDD/F sum as I-TEQ; (8) Sum of the following chlorobenzenes: chlorobenzene, 1,2 - Dichlorobenzene, 1,2,4 - Trichlorobenzene, 1,2,4,5 - tetrachlorobenzene, 1,3 - Dichlorobenzene, 1,4 – Dichlorobenzene.

Brazilian norm includes inorganic pollutants, such as arsenic, barium, selenium and molybdenum, not presented in other norms. All other compounds considered, with the exception of chromium, have similar limits as the one established by the US norm. The European Community norm is more restrictive than both, US and Brazilian norms, except for lead and chromium. Therefore the European Community Working Document on Sludge (WDS) is more restrictive for all inorganic compounds, and also includes some priority organic compounds in sludge.

A sample were considered as non-compliance when a chemical concentration were above the norm threshold. The frequency of non-compliances was calculated for each norm by SSHI range group. Three groups were defined within a unit interval $SSHI < 1$; $SSHI \geq 1$ and < 2 ; $SSHI \geq 2$ and < 3 ; the last one $SSHI \geq 3$ and < 5 , within two units interval as only one sample had a SSHI above 4. Based on the median value (2.1) two groups, $SSHI < 2$; $SSHI \geq 2$, were defined (table 5).

Table 5 – Frequency (%) of sewage sludge samples above chemical threshold from different norm for sludge agricultural use, by SSHI rank groups

Norm	Sewage Sludge Hazard Index (SSHI)					
	< 1 (n=4)	1 -- 2 (n=9)	2 --3 (n=8)	3 --5 (n=7)	< = 2 (n=13)	> 2 (n=15)
Brazil (1) ^a	0	0	0	71	0	33
US/EQ ^b	0	0	0	57	0	27
86/278/EEC ^c	0	0	25	57	0	40
WDS ^d	50	33	75	86	38	80

n = number of samples; (a) Brasil, 2006; (b) Council, 1991; (c) USEPA, 1993; (d) EC, 2000;

All sludge samples with SSHI below 3 were in compliance for sewage sludge agricultural use for both Brazilian and US norms. For EU norm two non-compliances were present in this range because of Cu and Ni concentration among samples with SSHI between 2 and 3. Generally the more restrictive the norm higher the frequency of non-compliances. This comparison needs to be looked upon carefully because some norms consider only a small set of pollutants, which could be the reason why some samples with high SSHI were still considered as compliance.

Samples with SSHI below 2 presented non-compliances only when compared to WDS thresholds. Cd (5 samples), Cu (1 sample) and PCB (1 sample) concentrations were accountable for this result. Still considering WDS norm,

samples with SSHI above 2 presented 80% of non-compliances due to the concentration of Cd (5 samples), Cu (3 samples), Cr (2 samples), Ni (3 samples), Zn (5 samples), PAH (3 samples), DEHP (1 sample) and PCB (1 sample). This increase of non-compliances for the most restrict criteria (WDS) indicates that the SSHI approach has a positive assertiveness in the early rejection of sludge samples intended to be used as soil amendment in agricultural land.

Those analyses of non-conformance by SSHI range groups were limited to the few compounds with threshold values available in sewage sludge norms. We know that several other chemical pollutants are present in this complex matrix (Clarke and Smith, 2011; Harrison et al, 2006), that can fully or partly migrate to the aqueous extract dissolved by water or others cosolvents, and has a synergic, additive or antagonic interaction with other compounds which could affect each bioassays. For this reason, the relationship found for the samples considered shows that a SSHI below 2 has lower probability to be fitotoxic or harm two screaner species sensitive to pollutants harmful to others organisms. Considering the precautionary principle, samples that scores a SSHI above 2 should be warranted or it's use avoided in agricultural land because the aqueous extract that represents the sample mobile fase led to toxicity, meaning a harzardous sample or a confounding factor in bioassay (Postma et al, 2001).

5 –CONCLUSIONS

The proposed SSHI, based on simple and low cost toxicity tests, seems to be a promising tool for assessing the degree of hazard of sewage sludge samples. If only the three proposed bioassays are performed for the characterization of a sewage sample, a SSHI below 2 seems to warrant the compliance with Brazilian, US and EU legal values. More data is required for a full validation of the proposed index.

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