Pollutant monitoring in sludge treatment wetlands

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ABSTRACT

Phragmites australis for sludge dewatering and stabilization processes have been widely proved. The presence of reeds, indeed, efficiently allows solids dewatering and organic matter stabilization in order to obtain a stabilised product that can be suitable for land application, even if its environmental impact has to be considered. The actual revision of the European Union’s Working Document on Sludge (2000), in fact, seems to be addressed to detect two principal categories of pollutants in sludge for agricultural use: heavy metals and toxic organic compounds. In this study are presented results about sludge stabilization and monitoring of heavy metal fractionation and organic compounds in four urban wastewater treatment plants managed by Acque S.p.A., (Tuscany, Italy). To evaluate the process of sludge stabilization parameters were determined that highlight the biochemical and chemico-structural properties of sludge organic matter. The results showed that stabilization of the sludge over time occurred as shown by the low content of water soluble carbon and dehydrogenase activity, and by the re-synthesis of humic-like matter highlighted by the pyrolytic indices of mineralization and humification. Results about fractionation showed that heavy metals were retained in fractions related to the stabilized organic matter. Moreover, toxic organic compounds showed a drastic reduction at the end of the monitoring period.

Key words | heavy metals, organic matter, pyrolysis-gas chromatography (Py-GC), sludge treatment wetland, toxic organic compounds (LAS, NPE, DEHP)

INTRODUCTION

The effectiveness of sludge treatment wetlands (TW) in treating and stabilizing sludge has been clearly proven (Nielsen 2003; Peruzzi et al. 2009, 2010; Uggetti et al. 2010), but the environmental impact of land application of the final product has to be taken into consideration. The actual revision of the European Union’s Working Document on Sludge (2000), in fact, seems to be addressed to detect two principal categories of pollutants in sludge for agricultural use: heavy metals and toxic organic compounds. Concerning heavy metals, many authors evaluated their environmental impact by determining not only their total content, but also their bioavailability and their capacity for remobilisation by chemical fractionation technique (Fuentes et al. 2004; Amir et al. 2005). On the other hand, considering toxic organic substances, the third draft of Sewage Sludge Directive (European Union’s Working Document on Sludge 2000) indicates some of the organic compounds that will have to be monitored: linear alkyl benzene sulfonates (LAS), nonylphenol and nonylphenol ethoxylates with 1 or 2 ethoxy groups (NPE), and di-2-ethylhexyl-phtalate (DEHP). The objective of this research was to determine the quality of organic matter sludge stabilised in TWs, and also their heavy metal bioavailability and the presence of toxic organic compounds, such as LAS, NPE and DEHP.

MATERIALS AND METHODS

Sludge treatment wetland

Dewatering and stabilization of sludges were investigated in four urban wastewater treatment plants of Acque S.p.A., WWTP 1 La Fontina, WWTP 2 Oratoio, WWTP 3 Colle di Compito and WWTP 4 Pittini, all situated in Tuscany Region (Italy). In this paper, results after 6 (period: March 2006 for La Fontina and Oratoio treatment wetlands;
September 2006 for Colle di Compito and Pittini treatment wetlands and 48 (October 2009 for La Fontina and Oratoio treatment wetlands; March 2010 for Colle di Compito and Pittini treatment wetlands) months are reported (Table 1).

The drying emergency beds inside the WWTPs were adapted and transformed into sludge treatment wetlands (TW). The beds were open (uncovered), the slope was 1% and each bed was provided with a drainage system made up of 2 layers of gravel with two different diameters: the bottom layer was 25 cm deep with gravel of 40/70 mm and the top layer was 15 cm deep with 5 mm gravel. Seedlings of *Phragmites australis* were planted at a distance of 50 × 50 cm during August 2005; the seedlings were irrigated, with water effluent coming from the wastewater plant to enhance plant rooting, maintaining a constant level of 5 cm of water over the ground surface until the first sludge application. The outflow from the drainage system was collected by gravity and then pumped back to the treatment plant for further treatment. Sewage sludge from conventional activated sludge was applied, every 2 weeks from autumn to spring and every week during summer, from October 2005 in TW 1 and TW 2, while in TW 3 and TW 4 from March 2006 (Table 1).

For each bed was taken five subsamples, which were mixed in order to obtain a representative sample of each bed. The samples were collected near the gravel layer. The plant material was removed from samples. About 20 days before the sampling, the sludge applications were stopped.

**Methods**

The following parameters related to different aspects are analyzed:

- **Sludge Mineralization and Humification:** Total organic carbon (TOC), total nitrogen (TN), water-soluble carbon (WSC), dehydrogenase (DHase), index of mineralization (O/N) and humification (B/E3) derived by pyrolysis–gas chromatography (Py-GC) analyses.

- **Sludge Biotoxicity:** heavy metal fractionation and toxic organic compounds, linear alkyl benzene sulfonates (LAS), nonylphenol and nonylphenol ethoxylates with 1 or 2 ethoxy groups (NPE), and di-2-ethylhexyl-phthalate (DEHP).

TOC and TN were analysed by RC-412 multiphase carbon and FP-528 protein/nitrogen (Leco). WSC was determined according to the method of Yeomans & Bremner with K$_2$Cr$_2$O$_7$ and H$_2$SO$_4$ oxidation (Yeomans & Bremner 1988). DHase activity was tested by the method of Masciandaro et al. (2000), using 2-p-iodo-nitrophenylphenil-tetrazolium (INT) as substrate. The dried sludges were put into pyrolysis microtubes in a platinum coil probe (CDS Pyroprobe 190) and pyrolysis was carried out at 800 °C for 10 s, with a heat gradient of 10 °C/ms. The probe was directly coupled to a

| Table 1 | Wastewater treatment plants and loading program |
|---|---|---|---|---|
| | TW 1 (La Fontina) | TW 2 (Oratoio) | TW 3 (Colle di Compito) | TW 4 (Pittini) |
| Population equivalent (p.e.) | 30,000 | 10,000 | 4,000 | 5,000 |
| Basin area (m$^2$) | 1,210 (11 beds) | 375 (5 beds) | 225 (5 beds) | 256 (6 beds) |
| Bed area (m$^2$) | 110 | 75 | 45 | 42 |
| Loading rate (kg dw m$^{-2}$ y$^{-1}$) | 38 | 45 | 67 | 67 |
| Sludge (% dw) | 1 | 1 | 1.5 | 1.5 |
| Treated sludge (m$^3$ y$^{-1}$) | 4,600 | 1,700 | 1,000 | 1,200 |
| Bed loaded (m$^3$ y$^{-1}$ bed$^{-1}$) | 418 | 340 | 240 | 200 |
| Loading rate (m$^3$ m$^{-2}$ y$^{-1}$) | 3.80 | 4.50 | 4.40 | 4.70 |
| Loading/Resting Autumn-Winter-Spring (days) | 1/14–20 | 1/14–20 | 1/14–20 | 1/14–20 |
| Loading/Resting Summer (days) | 1/7–10 | 1/7–10 | 1/7–10 | 1/7–10 |
| Sludge height 6 months (cm) | 5–10 | 10–15 | 5–10 | 10–15 |
| Sludge height 48 months (cm) | 25 | 35 | 35 | 40 |
Carlo Erba 6000 gas chromatograph with a flame ionization detector (FID). The pyrograms obtained were quantified by normalizing the areas of the selected seven peaks (acetic acid, acetonitrile, benzene, toluene, furfural, pyrrole and phenol). Identification of pyrogram fragments in the samples was carried out comparing the relative retention times to standard spectra (Ceccanti et al. 2007). For heavy metal fractionation the Community Bureau of Reference (BCR) method was followed (Mocko & Waclawek 2004). DEHP, NPE and LAS were simultaneously extracted from air-dried samples with methanol by microwave-assisted extraction (5 mL of methanol, 10 minutes and 250 W microwave irradiation; Villar et al. 2007). Chromatographic analysis was performed on an Agilent 1,100 series high performance liquid chromatograph (HPLC) with an ultraviolet diode array (λex = 226 nm) and fluorescence detectors (λex = 226 nm; λem = 501 nm). Organic compounds separation was carried out using a ZORBAX Eclipse XDB-C8 (150 × 3 mm i.d., 5 μm). As mobile phase acetonitrile and 10 mM ammonium acetate solution was used at different gradients (30:70 for 8 minutes, 65:35 for 7 minutes, 100:0 for 10 minutes and 70:30 for 5 minutes). A thermo-stated column at 28°C and a flow rate of 1 mL/minute were adopted (Santos et al. 2007; Pakou et al. 2009).

In order to understand the effectiveness of TW process in degrading toxic organic compounds, sewage sludge deriving from conventional activated sludge of each WWTP were analyzed as comparison.

Statistical procedures of the STATISTICA 6.0 software were used. Analysis of variance (ANOVA) was used to evaluate the differences (p < 0.05%) between time (6 months and 48 months) within the TWs. Principal component analysis (PCA) was applied to all results obtained during the experiments. The PCA is a multivariate statistical data analysis technique which reduces a set of raw data to a number of principal components that retain the most variance within the original data in order to identify possible patterns or clusters between objects and variables (Pardo et al. 2004).

RESULTS AND DISCUSSION

Sludge mineralization and humification

All results about sludge mineralization and humification are presented in Table 2. Even if TOC and TN content had not shown very significant changes during the time, the mineralization of organic matter was highlighted in all TWs, both by the impressive decrease of dehydrogenase activity and, by the significant decrease of WSC (Table 2). DHase, which represents the biological oxidation processes of microbial activity, and soluble form of carbon, in fact, reached values that kept metabolic activity of the sludge at low levels, thus suggesting that the organic matter is going towards a bio-stabilization status. All these results are comparable with values presented in previous papers (Giraldi et al. 2009; Peruzzi et al. 2009, 2010); in fact, WSC and DHase activity values resulted lower after 48 months of treatment with respect to values reported.

Py-GC enables the characterization of soil organic matter quality from a chemical-structural point of view. The index of humification B/E3 (benzene to toluene, the former deriving basically from condensed aromatic structures, the latter deriving from aromatic structures containing short aliphatic chains) increases when organic matter is becoming more mature. The index of mineralization O/N expresses the ratio between pyrrole (a heterocyclic aromatic organic compound derived from nitrogenous compounds, humified organic matter, and microbial cells), and furfural (a pyrolytic product coming from polysaccharides degradation). The higher the ratio, the higher the extent of mineralization of organic matter, meaning that high concentration of labile organic compounds is still present. The mineralization index (O/N) showed in all TWs a significant decrease, while the humification index (B/E3) increased, in particular in TW 1, TW 3 and TW 4. These results confirmed the effectiveness of biostabilization process occurring in TWs, in that as the labile organic matter is degraded the more stable one (with humic characteristics) is being formed during the time (Ceccanti et al. 2007); moreover, these values resulted higher with values reported in previous papers (Peruzzi et al. 2009, 2010).

Sludge toxicity

Heavy metal fractionation

The total heavy metal content (Table 3) remained below the level established by law for the reuse of sewage sludge in agriculture (Italian regulation, Decreto legislativo n° 99 del 1992, D.lgs. 99/92; Directive 86/278/EEC 1986).
The procedure for fractionation differentiated the sludge heavy metals into four fractions:

1. Exchangeable fraction associated with carbonated phase (Fraction 1). Metals are adsorbed on the sludge components and Fe and Mn hydroxides. This is the most mobile fraction potentially toxic for plants.

2. Reducible fraction associated with Fe and Mn oxides (Fraction 2). Heavy metals are strongly bound to these oxides but they are thermodynamically unstable in anoxic and acidic conditions.

3. Oxidisable fraction bound to organic matter (Fraction 3). It is well known that metals may be complexed by natural organic substances. These forms become soluble when organic matter is degraded in oxidising conditions. This fraction is not considered to be bioavailable and mobile because the metals are incorporated into stable high molecular weight humic substances, which release small amounts of metals very slowly.

4. Residual fraction (Residual Fraction). The residual solids mainly contain primary and secondary solids that occlude the metals in their crystalline structures. They are considered to be not extractable and in an inert form.

Results about fractionation showed that the sludge phytostabilization, activating the humification process, enabled heavy metals to link tightly with humified organic matter, thus making them less available in the environment after 48 months of treatment (Figure 1). In fact, most of all metals were linked to fractions less available to vegetation, such as Fraction 3 (bound to organic matter) and Residual Fraction (related to the mineral structure), which both increased significantly during the time. Other authors found higher content of metals associated with Fraction 1 and Fraction 2 in sewage sludge stabilised with traditional methods (thickening, heat treatments, anaerobic digestion, etc) (Walter et al. 2006; Fuentes et al. 2008).

### Toxic organic compounds

In order to understand the effectiveness of TW process in degrading toxic organic compounds, sewage sludge deriving from conventional activated sludge of each WWTP were...
analysed as comparison. The concentration of LAS found in all types of samples deriving from conventional activated sludge basins and from TWs at 48 months, was below the limit of concentration suggested by European Union’s Working Document on Sludge (2000), (2,600 mg/kg dw) for land application. Because of their relatively high biodegradability in the

Figure 1 | Heavy metal fractionation (%) in TW 1 at 6 (a) and 48 months (b), in TW 2 at 6 (c) and 48 months (d), in TW 3 at 6 (e) and 48 months (f), in TW 4 at 6 (g) and 48 months (h).
aerobic environment of reed bed systems, the LAS concentration after six months was dramatically decreased in all TWs, and after 48 months, the values were even lower (Figure 2(a)). NPE (the sum of: nonylphenol, NP; nonylphenol ethoxylate with 1 ethoxy group, NP1EO; and, nonylphenol ethoxylates with 2 ethoxy groups, NP2EO) resulted in higher concentration with respect to the limit of 50 mg/kg dw suggested in the draft of the working document of sludge (Figure 2(b)). The content tended to decrease significantly in all TWs after 48 months, thus demonstrating the presence of aerobic conditions (Fountoulakis et al. 2005); these conditions had also allowed the degradation of DEHP (Figure 2(c)), which has lipophilic properties (Aparicio et al. 2009), to a level lower than the limit concentration of 100 mg/kg dw. Moreover, the content decreased significantly over time, for the presence of aerobic conditions assured by the synergic action established by roots and microorganisms within beds. The presence of Phragmites australis, in fact, guarantees the transfer of atmospheric oxygen at root zone level (Brix et al. 1996).

Similar studies about the decrease of toxic organic compounds in sludge treatment wetlands and the importance of aerobic conditions for their degradation were found by Nielsen (2005); in fact, in this paper a higher reduction of hazardous organic compounds were found at surface and subsurface layers, where it is supposed to be higher oxygen diffusion.

**Statistical analysis**

The principal component analysis (PCA) is a multivariate statistical data analysis technique which reduces a set of raw data to a number of principal components that retain the most variance within the original data in order to identify possible patterns or cluster between objects and variables (Pardo et al. 2004; Fuentes et al. 2008). In order to minimize the number of variables, the contents of different metal fraction were transformed from mg/kg to meq/kg and then the content for each fraction was summed. The PCA of the data set indicated 75.5% of the data variance as being contained in the first three components (Figure 3(a)). PC1 was closely associated with WSC, humification index B/E3, Fraction 3 and Residual fraction, while PC2 was linked with dehydrogenase activity and toxic organic compounds; PC3 was associated with TOC, TN, and Fraction 1. The score plot (Figure 3(b)) provides a graphical representation of the TWs, identifying cluster of the objects with similar biochemical properties, over time; in fact, the graphical closeness of a variable with an object in the plot showed a clear correlation between them. All TWs had a similar behaviour over the time (Figure 3(b)): they were situated at the right of the plot at the beginning, and then they shifted towards PC1 and PC2 to the bottom left of the plot. At first, the TWs were linked with parameters associated to mineralization process (higher content of water soluble carbon and dehydrogenase activity, higher values of mineralization index O/N) and
toxic organic compounds (higher content of LAS, NPE, DEHP); while at the end of experimentation they resulted associated with humification index, Fraction 3 and Residual Fraction, being TWs characterized by higher content of heavy metals in Fraction 3 and Residual Fraction and more humified organic matter.

**CONCLUSIONS**

The study demonstrated the efficiency of the process of mineralization and humification of sludge organic matter occurred in all the four systems, thus highlighting the suitability of sludge TWs to different plant sizes (4,000–30,000 p.e.) and organic loading (38–67 kg dw/m² year).

The phytostabilization process is validated in consideration of three main aspects:

1. Progressive stabilization and humification of sludge organic matter, as shown by the decrease in enzymatic activity and soluble forms of carbon and by the opposite behaviour of pyrolytic indices of mineralization and humification.
2. High reduction of organic pollutants due to the aerobic metabolism insured by plant action.
3. Low bioavailability of inorganic contaminants as a consequence of the formation of organic matter-heavy metal complexes.

Finally, both organic matter stabilization-humification and detoxification process can be well monitored by appropriate integration of specific biochemical, chemical and chemical-structural parameters.

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