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ORIGINAL RESEARCH PAPER

MONITORING OF ORGANIC POLLUTION AND MATURITY OF ORGANIC MATTER FROM SLUDGE LANDFILLING

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Abstract: The biotransformation during the 3 years of sludge landfilling was evaluated by physicochemical analysis and phytotoxicity test. The final product exhibited a high degree of decomposition rate (51.06 %) than the controls as shown by a decrease of C/N ratio of about 19.67. The results showed that the lipid, surfactant and polyphenol as main compound of the sludge were breakdown over time. The concentrations decreased from 29.9 to 11.8 $mg \cdot g^{-1}$ and 3.4 to 0.6 $mg \cdot g^{-1}$, respectively for surfactant and polyphenols after 3 years of landfilling. This corresponds to a reduction of 80.2 % for polyphenols and 60.4 % for surfactant, due to the microorganisms activity. Total lipids decrease from 16.5 to 6.27 $mg \cdot g^{-1}$ of dry matter, representing an abatement rate of about 62 %. The evolution of organic matter reflects the progress of the humification process, which judging by the increase in the polymerization degree, is about 20 %. The landfilling efficiency to reduce phytotoxicity of sludge was confirmed by the germination index, which reached 52 and 59 %, respectively for alfalfa and cress after 3 years of landfilling. These results are promising and pave the way for agricultural spreading of sludge.

Keywords: *landfilling, phytotoxicity test, polyphenol, sludge surfactant, total lipids*

INTRODUCTION

In recent decades Morocco has experienced a significant increase in volume of wastewater. The potential of wastewater in Morocco was estimated at 500 million m^3 in 2000 and reach 900 million m^3 in 2020, according to the Moroccan Ministry of Agriculture and Fishing. To consolidate achievements and address the above challenges, the development strategy of the 2009 water sector in line management and development of the supply has set a target for 2030, three hundred (300) million m^3 / year of treated wastewater reuse in watering golf courses and green areas and irrigation of crops that surface there. However, the purification system adopted for the wastewater treatment generates significant amounts of sludge, estimated in 2010 to 40,000 tons per year, and the forecast are for 300,000 tons per year in 2025, as mentioned in the national report management wastewater treatment plant (WWTP) Strategy sludge in Morocco in 2009.
Sewage sludge will also remain a product the quality of which is not strictly controllable, which may have no secure long-term outlet and which usually entails processing, transport and disposal costs of about half the total cost of operating the sewage works. Sludge is thus often regarded as the major problem of water pollution control. In recent years, sewage sludge has become an international topic with numerous conferences and, in the case of the European Union (EU), interstate coordinated research and scientific committees focusing on various common problems [1]. There are several solutions for sludge disposal, but the choice depends on the cost of the installation, the origin of the sludge, and the potential impact of the sludge if it is discharged directly into the environment without any treatment [2].
Lesieur Cristal company, which is the industry leader in Morocco for the production and marketing of edible oils, pure oils, olive oils and products aids (soap, distilled fatty acids) has invested in the treatment of their wastewater discharges through the (WWTP) with a processing capacity of 1850 m^3 per day. The treatment plant is currently running at about 79 % of its capacity rate that of about 1450 m^3 per day. The sludge produced during treatment with the SBR (Sequencing Batch Reactor) WWTP, is estimated at 7 tons / day, this by-product is stored in the discharge of the Lesieur Cristal Company. Over time, these releases are biodegraded naturally and can then be used in agricultural soil, after checking their free load of pollutants. The decomposition of organic matter and the reduction of the toxic effect by the landfilling of the sludge is a natural way of recycling that allows the decomposition of biodegradable organic waste, in particular sewage sludge. The present study is primarily concerned with the monitoring of sludge quality during landfilling. Firstly this will provide some information about the stability and the effect on various organic compounds in the sludge from oil refining as well as their degree of biodegradation and their impact on the plants growth, and secondly this will allow us to better assess the effectiveness of sludge landfilling and disposal effects of pollutants, which opens the way to agricultural reuse.

MATERIALS AND METHODS

Sampling and sludge landfilling

The sludge (T0= raw sludge, T1=6 months, T2=18 months, T3=24 months, and T4=36 months of landfilling) comes from WWTP of company of edible oils in Casablanca. Homogeneous samples (1 kg) were obtained by careful mixing of several sub-samples taken at different points (height and depth) of the discharge and quartering them. The samples were stored at -20 °C before analysis. For each analysis three replicates were made.

Physico-chemical analyses

The *p*H and the electrical conductivity were measured on an aqueous extract of the sludge at room temperature (1 g / 10 mL of distilled water). Moisture contents were determined by drying 100 g of sludge at 105 °C for 48 h [3]. Total organic carbon and ash content were calculated after calcination in furnace at 600 °C for 6 h. Total Kjeldahl nitrogen (TKN) was assayed in 0.5 g samples by using classical Kjeldahl procedure, by steam distillation according to AFNOR [4]. The decomposition rate (DR) was calculated using the following formula [5]:

$$\text{Decomposition Rate } \% = 100\text{-}100\ [Ash_i\ (100\text{-}Ash_f)]\ /\ [Ash_f\ (100\text{-}Ash_i)] \qquad (1)$$

where Ash_i is the initial level of ash, and Ash_f is the final level.

Extraction of total lipids and polyphenols

Polyphenols were extracted with methanol and purified with ethyl acetate by the method described by Macheix *et al.* [6]. The assay was performed according to the Folin-Ciocalteu method.

Total lipids were extracted with a mixture of methanol/chloroform (v/v; 1/2) and calculated according to the method of Folch [7].

Extraction of humic substances

The humic acid was extracted from a fresh sample 30 g. It was treated three times with 40 mL of distilled water so as to extract the water-soluble non-humic substances (sugars, proteins, etc.). Then, the humic substances were extracted with 40 mL NaOH (0.1N). This was repeated several times until the extract obtained was colorless. After filtration, the solutions were pooled and the humic acids were separated from fulvic acids after being precipitated out of first fraction (humic acid) with H_2SO_4 pH around 1-2 for 24 h at 4 °C; the content of each fraction was determined by the $KMnO_4$ oxidation method.

Extraction of surfactant

Surfactant contents were extracted using toluene. The assay was performed according to the colorimetric method of Jones [8].

Phytotoxicity test

To determine the germination index (GI), the seeds of a few significant plant species such as cress and alfalfa were tested in 5 mL of water-soluble extracts of sludge (from 10 g of fresh sample in 100 mL of distilled water) in the darkness at room temperature (25 °C) for 72 h [9]. The germination test was conducted in Petri dishes. Three replicates were made. The phytotoxicity test was computed as the product of the percentage of viable seeds. It was performed by monitoring the seedlings emergence, the number of germinated seeds (tests 24 h), and growth of roots (after 72 h), using the following equation:

$$GI\ \% = (NG_{ext} \cdot LR_{ext}) / (NG_{water} \cdot LR_{water}) \cdot 100 \qquad (2)$$

where NG_{ext}, NG_{water} = number of seeds germinated in water soluble extracts and distilled water, respectively; and LR_{ext}, LR_{water} = the length of rootlets in soluble extracts and distilled water, respectively.

Statistical analyses

The variations occurring in the physicochemical parameters during landfilling of the sludge were studied using principal components analysis (PCA), which was applied on the matrix of correlation between the variables. The statistical treatments were carried out using the software SPSS Win version 10.

RESULTS AND DISCUSSION

Physicochemical characterization

The results for the fresh sludge are shown in Table 1. The raw sludge is characterized by an acid *p*H of 5.25, with a high level of undesirable elements such as lipids, polyphenol and surfactant. According to Abouelwafa *et al.* [10], Zaim *et al.* [11] the sludge produced at the treatment plants of refineries effluent of crude oil are generally characterized by an acidic *p*H, an electrical conductivity from 1.20 to 1.80 $mS \cdot cm^{-1}$ and a total nitrogen from 0.72 to 1.50 %. Nevertheless, the sludge is rich in organic carbon and poor in nitrogen, hence the C/N ratio is greater than 32 (Table 1). Loudini, [12] shows that the organic carbon content is around 40 % and the C/N ratio varies from 60.71 to 27.76 %. Instability of sludge parameters is influenced by the variability of release refineries, which are not constant in time.

***Table 1**. Physical and chemical characteristics of the fresh sludge*

Parameters	Average
pH	5.25 ±0.08
Conductivity [$mS \cdot cm^{-1}$]	1.2 ±0.6
DM %	44.3 ±1.5
OM [%] (DWt)	84,06 ±2.3
Total lipids [$mg \cdot g^{-1}$] (DWt)	16.5
NTK [%]	0.925 ±0.5
TOC [%]	30.1 ±1.5
C/N	32.5
Polyphenol [$mg \cdot g^{-1}$] (DWt)	3.455
Surfactant [$mg \cdot g^{-1}$] (DWt)	29.92

DWt: Dry Weight; TOC: Total Organic Carbon; KTN: Kjeldahl Total Nitrogen, OM: Organic Matter, DM: Dry Matter

The results of this study Indicate that sludge from the WWTP Lesieur Cristal Company have a very high rate of dry matter 44.3 % (Table 1). These results are explained by the fact that WWTP has mechanical dewatering equipment. After 3 years of landfilling sludge dry matter increased to reach 90 % (Table 1) that give a consistence character to the sludge.

The result of the evolution of organic matter by calcination method shows a decomposition rate around 51.06 % after 3 years of landfilling.

Table 2. *Physicochemical parameters of landfilling of the sludge*

Sludge landfilled	DM [%]	OM [%]	C/N	DR [%]
T0	44.3	84.06	32.5	-
T1	67.08	81.56	24.03	16.07
T2	81.59	78.44	20.69	30.98
T3	78.81	77.43	19.99	34.91
T4	90.18	72.07	19.67	51.06

T0 =raw sludge, **T1**=6 months, **T2**=18 months, **T3**=24 months, and **T4**= 36 months, **OM**: Organic Matter; **DM**: Dry Matter; **DR**: Decomposition Rate

Two distinguished phases with a different decomposition rate was determined (Table 2). Rapid decomposition phase (T0 to T2) characterized by a rapid decomposition rate of about 30.98 %, and 20.08 % for a slow phase (T2 to T4). That is due to the high concentration of recalcitrant compounds in sludge such as lipid, polyphenol, and surfactant, identified in this study. Many studies reported the succession of degradation of organic compound with different proportion rate [2, 13, 14]. The same authors show that the intense microbial activity resulting from the degradation of the simple molecules present in the substrate characterized by a high rate is followed by a second phase which is characterized by the exhaustion of easily metabolized organic compounds from the medium with only compounds resistant to degradation remaining (e.g., lipid, phenol, lignin, cellulose). EL Fels et al. [15] show that the high recalcitrant compounds degradation such as lignin is due to mineralization and biodegradation, which is favored by the co-composting conditions (humidity, temperature and the presence of decomposer microorganisms.

The C/N ratio decreased from 32.5 to 19.67 after 3 years of landfilling (Table 2). The high value of C/N ratio can be attributed to the absence of nitrogen substrate, as they are characteristics of a product containing recalcitrant organic matter [16]. In fact, in Biocycle guide, [17] is confirmed that this report must be typical at the pace of Obsessive Compulsive Disorder (OCD) (microorganisms consume 15 to 30 times more carbon than nitrogen). This is because these organisms require organic carbon and nitrogen NTK as an energy source instead of carbon dioxide for plants [18].

Total lipids

The complete biodegradation of total lipids comprises of two successive steps: lipolysis (release of fatty acids from glycerides) and the oxidation of these fatty acids. For a high removal rate of fatty compounds of sludge by a different indigenous microflora, a both steps are needed to ensure hydrolyze triglycerides and subsequent oxidation of fatty acids. According to these above reasons, total lipids were degraded during sludge landfilling, with an abatement rate of 62 % (Figure 1).

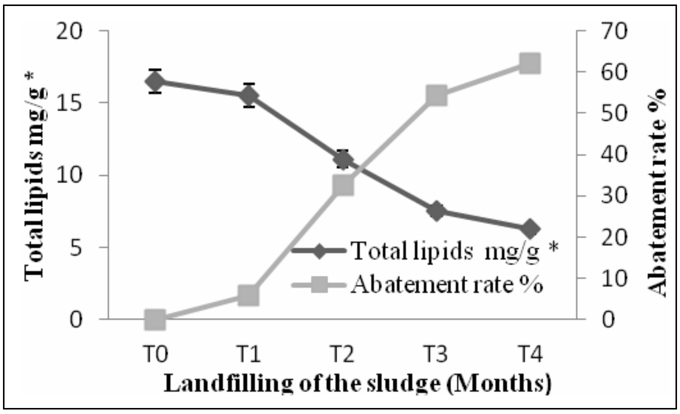

Figure 1. *Evolution of total lipids during landfilling of the sludge*
T0= raw sludge, **T1**=6 months, **T2**=18 months, **T3**=24 months, **T4**= 36 months
* **DWt**: Dry Weight

This low percentage of degradation compared to other authors [2, 10], is likely due to the heterogeneous composition of these fatty residues, which are constituted of feed fat, and mineral oil.

Humification process during sludge landfilling

The evolution at versus time of humic and fulvic acid during landfilling of oily sludge is presented in (Table 3). The content of humic substances increased from 15.17 to 19.38 $g \cdot kg^{-1}$ DWt during the sludge landfilling for 3 years. That is due to the humification process. Humic substances (HS) are the major fraction of organic matter because of their effect on soil ecology, the structure, fertility, and plant growth [19]. A portion of the organic material in the sludge was mineralized during landfilling, but the residual organic materials are transformed into new organic materials such as humic substances, which are produced by the process of humification [20].

Table 3. *Humification process during landfilling of the sludge*

Sludge landfillied Time [Month]	HA [$g \cdot kg^{-1}$] /DWt	FA [$g \cdot kg^{-1}$] /DWt	PD [%]
T1	9.85	5.32	185.1
T2	16.59	7.91	209.7
T3	12.66	6.05	209.2
T4	13.36	6.02	222

HS: humic substances; **HA**: Humic acid; **FA**: fulvic acid; **PD** (Polymerization Degree) = HA/FA * 100.
T1=6 months, **T2**=18 months, **T3**=24 months, **T4**= 36 months
DWt: Dry Weight

Several studies have shown that increasing of HS is an indicator of the degree of organic matter humification, and therefore the degree of maturity [2, 19]. Huang et al. [19] explained that the humification of organic matter occurs mainly through the humic acid (HA) fraction and little through the fraction fulvic acid (FA). Thus, the ratio HA/FA has often been proposed as an indicator of the humidification process and as maturity index [21]. Jouraiphy et al. [22] showed that the increasing of the ratio HA/FA resulted in the formation of HS by polymerization or degradation of non humic substances, followed by the formation of humic polycondensed structures.
The significant increase for polymerization degree (PD) seems to be the most sensitive index for humification process (20 %). As reported by several authors, El Fels et al. [2],

Sanchez et al. [21], Jimenez et al. [23], Vergnoux et al. [24] the increase in PD ratio can be explained by the formation of complex molecules (humic acids), the polymerization of simple molecules (fulvic acids), or by the biodegradation of non-humic compounds.

Surfactant evolution

The surfactant concentration of sludge landfilling decreased significantly after 3 years. That is influenced by several parameters, such as their content in the sludge, the treatment process (aerobic, anaerobic), the water hardness, and the age of the sludge after landfilling.
The biodegradation process is a significant environmental consequence on the decrease of the surfactant in the sludge. It can be noted that the reduction of surfactant was approximately 60.4 % after 3 years (Figure 2).

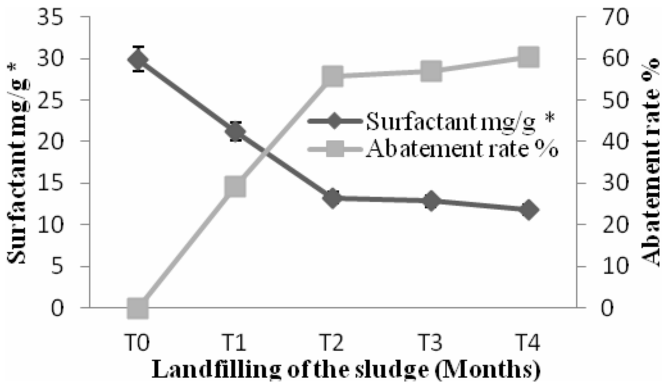

***Figure 2.** Evolution of surfactant during landfilling of the sludge*
T0= raw sludge, **T1**=6 months, **T2**=18 months, **T3**=24 months, **T4**= 36 months
* **DWt**: Dry Weight

A certain number of studies have shown that the aerobic treatment is an important solution for reducing the level of surfactant [25, 26]. Prats Perez et al. [27], Laguardia, [28] showed that the composting of the sludge results a reduction of surfactant concentration. Under aerobic conditions, some surfactant compounds will be degraded by ω-oxidation followed by β-oxidation in subsequent degradation of the alkyl chain. The degradation will be influenced by the availability of oxygen [29]. Generally in untreated soils, surfactant content is normally less than 0.2 $mg \cdot kg^{-1}$ DWt. Although sewage sludge, is the main source of surfactant in soil [30].

Polyphenol evolution

The degradation of polyphenol was very important during the first 18 months, with a reduction of 80.2 % then it stabilizes (Figure 3). This degradation can be explained by the conversion of phenols to oligomers and humic type of polymers. That leads to reduce their ability to be assimilated and their phytotoxicity. These results can be explained by means of the decomposition of organic matter: it starts with the hydrolysis of proteins, urea and soluble hemicellulose releasing ammonia, amino acids, sugars and aliphatic acids. During the decomposition progress, lignin and cellulose are degraded. Sugars, aliphatic and some polyphenols are used, with ammonia, for the synthesis of the microbial biomass [2].

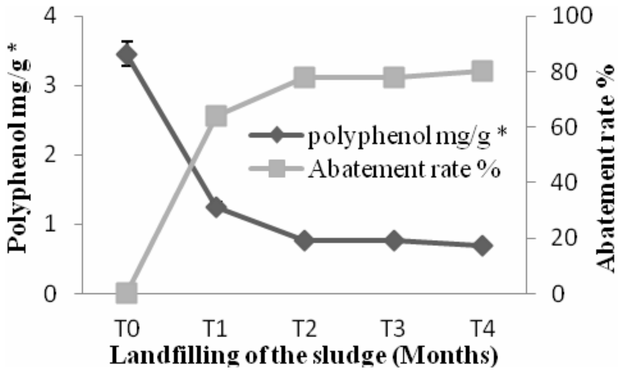

Figure 3. *Evolution of poly phenol during landfilling of the sludge*
T0= raw sludge, **T1**=6 months, **T2**=18 months, **T3**=24 months, **T4**= 36 months
* **DWt**: Dry Weight

In the presence of secondary metabolites of decomposing bodies, polyphenolic compounds can start auto-oxidation or be oxidized enzymatically, and condense to form humic substances [13].

Phytotoxicity testing

The water extracts of oily sludge landfilling for 6 months, showed very low germination index (GI), 24 and 13 % respectively for Cress and alfalfa (Figure 4), which shows a greater sensitivity to toxic compounds. After the sixth month of sludge landfilling, there was a significant increase in the germination index (intense degradation phase of organic matter), which reached 38 and 34 % respectively for Cress and alfalfa. The high values (52 and 59 %) of the GI recorded after 3 years can usually be explained by a strong reduction of phytotoxic substances such as lipids, polyphenol and surfactant, whose level fell after 3 years of sludge landfilling.

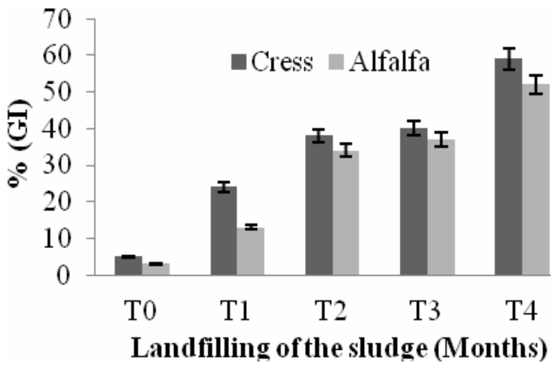

Figure 4. *Phytotoxicity variation during landfilling of the sludge*
T0= raw sludge, **T1**=6 months, **T2**=18 months, **T3**=24 months, **T4**= 36 months
GI: Germination Index

Statistical analysis

The results of the PCA run between the physicochemical parameters (Total lipids, surfactant, polyphenols, OM, DM, C/N, and Humic substance) during landfilling of the sludge are reported in Figure 5.

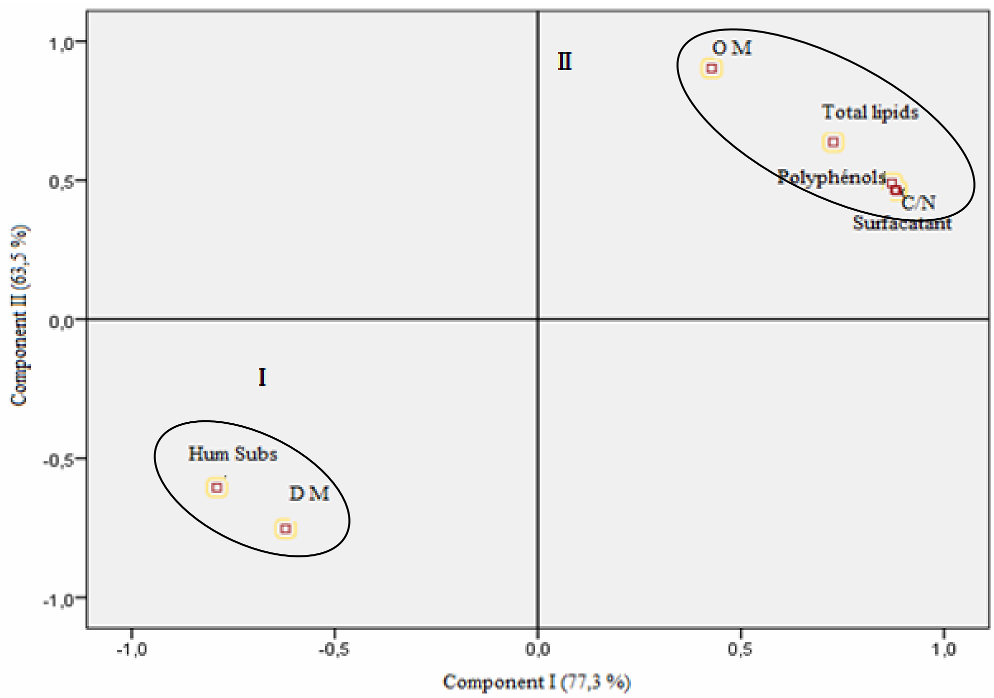

Figure 5. *Diagram of the ACP components of different parameters during landfilling of the sludge*
DM: Dry matter; **OM**: Organic matter; **Hum Subs**: Humic substance

Only two components of the PCA account for almost all the information or variability between different parameters, the first component explains 77.3 %, and the second component explains 63.5 % of the variability. The principal component is divided into two inversely correlated domains. The first one gathers the DM, and humic substance, which are closely correlated, for they evolve similarly as they increase during the landfilling of the sludge. The second domain consists of total lipids, C/N, OM, polyphenol, surfactant, and total lipids, which decrease during 3 years of landfilling of the sludge. The results showed a high correlation between different parameters, which shows the progress of the sludge landfilling.

CONCLUSIONS

The sludge landfill for 6, 18, 24 and 36 months have a lower evolution of nitrogen relative to the total organic carbon. In fact, after 3 years of landfilling, the C/N ratio decreased from 32.5 to 19.67. The rate of decomposition of organic matter reached 51.06 %. The result has shown that the different types of greasy residues could be partially removed, by landfilling sludge without any additional costs. The degradation of recalcitrant compounds was important during 36 months of sludge landfilling, with an abatement rate of 62, 80.2 and 60.4 % respectively for total lipids, polyphenol and surfactant. This reduction of sludge toxicity can be explained by the germination index, which reaches 50 % after 3 years of sludge landfilling.
The sludge landfilling seems to be a good way for reduction of toxics compounds, however the long time of storage to reach a high abatement rate disadvantage this type of treatment.

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