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TECHNICAL ARTICLE

Characterization and Toxicity Assessment of Wastewater from Rock Phosphate Processing in Tunisia

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Abstract Phosphate ore processing wastewater (WWPP) from the Gafsa phosphate region of Tunisia was characterized. The WWPP had a very high turbidity, an almost neutral or slightly alkaline pH, and high salinity. The average chemical and biochemical oxygen demands (COD and BOD) met wastewater discharge standards, but the COD/BOD5 (4.34) significantly exceeded biodegradability values. Total nitrogen, residual phosphorus, and some others chemical constituents exceeded wastewater discharge standards. Microbiological enumeration showed that the effluents were very low in microflora. Untreated WWPP and diluted (WWPP/4) inhibited bioluminescence of *Vibrio fischeri* by 76 and 45%, respectively. The WWPP had a phytotoxicity rate of 20–70%, respectively, for alfalfa and tomato seeds. Adding the effluent to soil for 60 days reduced the residual phytotoxicity of the WWPP-irrigated soil to about 15 and 34%, respectively, for tomato and alfalfa seeds.

Keywords Waste water · Phosphate industry · Salinity · Toxicity · Soil

Introduction

The phosphate industry is the cause of many environmental problems (Lipfert et al. 2000; Pope and Burnett 2002). Natural phosphates contain many metallic elements, some of which can negatively affect the environment (Jarvis and Burnett 1994). Gnandi and Tchangbedji (2006) observed that some diseases, such as dental fluorosis in children, are strongly related to the fluoride in the phosphate rock. Also, fine phosphate particles generated by mining have been found to negatively affect the human respiratory system (Pope and Burnett 2002). McDonnell et al. (2000) showed that they contribute to the incidence of cancer, while others have found that they can cause respiratory diseases, such as bronchitis and asthma (Herich and Holscher 2002).

With over a century of experience in mining, upgrading, and marketing Tunisian phosphates, the Company of Phosphates of Gafsa (CPG), is one of the world's leading phosphate producers (Narasiah et al. 1988). Production exceeded 8 million metric tons (t) in 2010, making CPG the world's fifth largest phosphate producer (Tijani and Fakhfakh 2011), though social unrest in Tunisia has subsequently affected production. The CPG works 10 surface mines in five phosphate fields, all in the same Eocene geological level, principally based around deposits in the Gafsa basin in southern Tunisia (Tijani and Fakhfakh 2011). The phosphate is 'washed' in several phases, including mechanical separation and treatment, to increase the P_2O_5 content (Narasiah et al. 1988). This consumes 5 t of water per ton of phosphate. However, of these 5 t of water, 3.65 t were recycled, 0.15 t accompanies the phosphates product as moisture, and 1.2 t are lost (Narasiah et al. 1988).

Thus, production of 8 million tons per year of marketable phosphate consumes approximately 10.5 million tons of waters (Tijani and Fakhfakh 2011). Moreover, the water that is being used by CPG is paleowater from Infill Continental, and is not renewable (Tijani and Fakhfakh 2011). In this study, we characterized CPG's wastewater from phosphate ore processing (WWPP) and investigated its effects on the receiving environment, in particular the soil, in order to develop a suitable water treatment process.

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Materials

Wastewater samples were collected from phosphate ore processing plant at Metloui-Gafsa, in southwestern Tunisia in February 2016 ($WWPP_A$), March 2016 ($WWPP_B$), and April 2016 ($WWPP_C$). The wastewater was sampled at the washing plant discharge (Fig. 1) and the samples were kept at −4 °C until analysis.

Soil samples were taken from uncultivated land in the Sfax region in southern Tunisia (north latitude 34°3′, east longitude 10°20′), where the mean annual rainfall is 200 mm.

Methods

WWPP Physicochemical Analyses

The pH was measured using a STARTER 2100 pH meter and the electrical conductivity (EC) was measured by a Cond 1970i conductivity meter. Probes were rinsed with distilled water before measurements. The turbidity was measured using a VTV turbidimeter.

The chemical oxygen demand (COD) was determined according to Knechtel (1978) in a BLOBLOCK 10120 reactor at 150 °C in the presence of an excess of potassium dichromate in an acidic medium. 2.5 mL of diluted sample was mixed with 1.5 mL of the potassium dichromate solution (10.215 g $K_2Cr_2O_7$ + 8.5 g $HgSO_4$ + 250 mL of H_2SO_4 (18 M), 1000 mL water) and 3.5 mL of H_2SO_4 (18 M) containing 10 g/L of $AgSO_4$. After 2 h of incubation, the optical density was measured at 600 nm. Due to the presence of salts in the WWPP, the COD was determined using Mohr salt solution (N/40) in the presence of ferroin as a color indicator.

The amount of suspended matter (SM) contained in the wastewater was determined by centrifugation at 4000 rpm for 20 min. The obtained pellet was collected in a porcelain crucible previously dried at 105 °C. Then, the centrifuge jar was rinsed with distilled water and that water was deposited in the same crucible. Finally, the crucible was again dried at 105 °C until the mass was constant. The dry matter (DM), which represents all of the organic and inorganic substances in solution or in suspension, was similarly determined by evaporation at 105 °C in a crucible until the mass was constant. The amount of volatile matter (VM) was defined as the difference between the dry matter and residue (ash) from the sample calcination.

Fig. 1 Wastewater outlet of the phosphate washing plant of the Company of Phosphate of Gafsa (CPG), Tunisia (sampling site)

Biological oxygen demand (BOD_5) was defined as the amount of dissolved molecular oxygen needed for oxidative degradation of the sample's biodegradable organic matter over 5 days at 20 °C and in the dark (to prevent algal growth). BOD_5 was determined by the manometric method with a respirometer (BSB-Controller Model 620 T WTW).

Total and ammoniacal nitrogen were determined according to Kandeler (1995). Organic nitrogen was defined as the difference between total nitrogen and ammoniacal nitrogen. Concentrations of Fe, Cu, Zn, Cd, and P were determined by atomic absorption.

WWPP Microbiological Analyses and Phytotoxicity.

Bioluminescence bacterial tests have become particularly popular because they are rapid, reproducible, simple to use, and unambiguous, and cause no ethical problems (Hernando et al. 2006). We measured microtoxicity by the bioluminescence inhibition (BI %) of *Vibrio fischeri* LCK480 using the LUMIStox system (Dr. Lange GmbH, Germany), according to ISO 11348-2 (1998). The inhibition of bioluminescence was achieved by mixing 0.5 mL of PMW and 0.5 mL luminescent bacterial suspension. The toxicity of the WWPP was expressed as the percent of BI relative to a non-contaminated reference. A positive control (7.5% NaCl) was included for each test (Aloui et al. 2007).

Total cultivable microfloras are microorganisms that grow in ordinary environments. To count this microflora, the LB medium is used. The dishes are seeded and incubated at 30 °C for 48 h and the results are expressed as CFU mL^{-1} (Gerba and Rose 2003). For sporulant bacteria, the sample was heated at 80 °C for 10 min. The dilutions were plated onto LB medium and the dishes were incubated at 30 °C for 24 h. Total coliforms were counted on TTC medium. The dishes were incubated at 37 °C for 72 h and the dark red colonies were counted.

The WWPP phytotoxicity was evaluated by determining the germination indexes (GI) of tomato (*Lycopersicumesculentum*) and alfalfa (*Medicago sativa*) seeds, using the method of Zucconi et al. (1981). The results were compared with a control that was irrigated with pure water.

Soil Analysis

The paste pH and EC of soil irrigated with WWPP and control soils were determined according to Sierra et al. (2001). Soil water content, dry matter, mineral matter, and organic matter content were determined using the methods of Mekki et al. (2014). The Kjeldahl method was used to determine total nitrogen and ammoniacal nitrogen (Kandeler 1995). Cultivable microfloras, sporulant bacteria, total coliforms, and fungi were enumerated according to Mekki et al. (2006).

Statistical Analysis

For each analyzed parameter, 5 repetitions were made for both the WWPP samples and soil samples. Data were subjected to analysis of variance (ANOVA) using the statistical system (SPSS Inc., IL, USA, version 16.0). Mean values were compared using the least significant difference test at $P \leq 0.05$.

Results and Discussion

WWPP Physicochemical Characterization

Table 1 Physico-chemical characteristics of sampled wastewater from phosphate ore processing (WWPP) (averaged values of 5 repetitions for each analysis)

Characteristics	$WWPP_A$	$WWPP_B$	$WWPP_C$
pH (25 °C)	7.35[a]	7.45[a]	7.48[a]
EC (dS m^{-1}) (25 °C)	9.37[a]	10.02[b]	9.55[a]
Turbidity (NTU)	842.5[a]	472.6[b]	514[c]
COD (mg L^{-1})	59.6[a]	67.5[b]	57.5[a]
BOD5 (g L^{-1})	15[a]	10[b]	25[c]
COD/BOD5	3.97[a]	6.75[b]	2.3[c]
Suspended matter (g L^{-1})	23[a]	14.5[b]	11[c]
Dry matter (g L^{-1})	25.8[a]	17[b]	14.6[c]
Organic matter (g L^{-1})	8.8[a]	6.7[b]	4.9[c]
Mineral matter (g L^{-1})	17[a]	10.3[b]	9.7[c]
Total Nitrogen Kjeldahl (mg L^{-1})	29.4[a]	23.2[b]	14.6[c]
Ammoniacal nitrogen (mg L^{-1})	18.4[a]	14.9[b]	8.7[c]
Organic nitrogen (mg L^{-1})	11[a]	8.3[b]	5.9[c]
P (mg.L^{-1})	23.04[a]	37.14[b]	22.03[a]
K (mg.L^{-1})	1374[a]	1238[b]	1181[b]
Ca (mg.L^{-1})	1290[a]	1070[a]	827[b]
Cu (mg.L^{-1})	1.24[a]	0.9[b]	0.4[c]
Zn (mg.L^{-1})	1.37[a]	1.1[a]	0.35[c]
Fe (mg.L^{-1})	679.4[a]	323.6[b]	108[c]
Cd (mg.L^{-1})	1.87[a]	1.42[a]	0.36[b]

Standard deviation <5%; means followed within the same row by the same small letter are not statistically different

The WWPP turbidity averaged about 609 NTU (Table 1), which far exceeds the allowable standards for groundwater recharge using wastewater (INOORPI. NT.106.03; WHO 2004). The WWPP had neutral or slightly alkaline pH values, due to their high calcium and phosphorus contents (Table 1). The pH meets the appropriate standards (INOORPI.NT.106.03; WHO 2004). However, the effluents had a high salinity (EC averaged 9.64 dS m^{-1}), indicating a serious risk of salinization of the receiving medium (Mekki et al. 2015).

The COD of the WWPP averaged 61.53 mg L^{-1}; the BOD_5 averaged16.66 mg L^{-1} (Table 1). These values meet the standards required of wastewater discharges (INOORPI. NT.106.03; WHO 2004). However, the ratio of COD/BOD_5 was high, reflecting its low biodegradability (Khoufi et al. 2006). This is due to its low BOD_5 values and indicates that physical–chemical treatment may be the appropriate treatment process for this wastewater (Yidana and Yidana 2010).

Determination of SM is very important in assessing the quality of discharged effluent and the appropriate treatment method (Rodier et al. 2005). Table 1 shows high levels of SM (16.16 g L^{-1}), which greatly exceeds the Tunisian standard for the discharge of wastewater (0.03 g L^{-1}). Likewise, most of the DM in the WWPP was in mineral form (MM = 12.33 g L^{-1}); the organic content was low (OM = 6.8 g L^{-1}). This confirms the dominance of minerals in this effluent (Ledesma-Ruiz et al. 2014).

The total nitrogen content averaged 22.4 mg L^{-1}, which exceeds the appropriate wastewater standard (15 mg L^{-1}; INNORPI.NT 106.03.1989; WHO 2004). In addition, mineralized N was more prominent that organic N in these effluents (Stigter et al. 2006).

The residual phosphorus analyses showed the wealth of WWPP in this element. The P content averaged 27.4 mg L^{-1} (Table 1), which greatly exceeds the Tunisian wastewater standards (15 mg L^{-1}). This high P level presents a risk of soil contamination and waterway eutrophication (Hammond and Day 1992; Sharifi and Safari 2012).

The WWPP are very rich in K, Ca, and Fe (Table 1). Their Cd content exceeds wastewater discharge standards, while the Zn and Cu levels barely meet them (INNORPI. NT 106.03.1989; WHO 2004). Elevated metal levels are not unusual for phosphate deposits (Golterman and De Groot 1994; Narasiah et al. 1988).

Microbiological Characteristics

The microbiological analyzes conducted focused on the detection and enumeration of total aerobic microflora

(TAM), fungi (F), sporulant bacteria, and total coliforms (TC), whose presence generally indicates contamination (Sridhara et al. 2008). The TAM count shows that the WWPP are very poor in this microflora (10^4 CFU mL^{-1}). The WWPP are also free of fungi, sporulant germs, and TC. The low BOD_5 value mentioned above reflects the recalcitrance of these wastewaters, and helps explain the low presence of microorganisms in this effluent (Khoufi et al. 2006).

Toxicity Assessment

Toxicity evaluation is an important parameter in waste characterization (Novotny´ et al. 2006). Many types of bioassays using representatives from microorganisms, plants, invertebrates, and fish are available (Tsui and Chu 2003). The WWPP caused an average *Vibrio fischeri* BI % of 76%. Even after the WWPP was diluted fourfold (WWPP/4), it had a residual BI (%) of 45%.

With respect to the WWPP's phytotoxicity, the average germination index for the three samples was about 30%, reflecting a phytotoxicity of 70% (Fig. 2). Alfalfa seeds were less sensitive to this waste water; their average GI% reached 80%, reflecting a 20% phytotoxicity compared to the control medium (pure water). So, besides the phytotoxic potential of this wastewater, it can be concluded that germination in the presence of this effluent depends on the species studied (Rusan et al. 2015; Saeedi et al. 2010).

Effects of the WWPP on Soil Characteristics

The studied soil was sandy at the surface and at depth, and poor in organic matter, with a slightly alkaline pH and low EC. Nitrogen, potassium, and phosphorus content were all very low. The effects of WWPP on some physicochemical, granulometric, and microbiological soil parameters were investigated for 60 days (from February 20 to April 20, 2016) at ambient conditions, and compared with control soil irrigated with pure water (Table 2). Since the WWPP was initially alkaline (pH 7.42), the pH values for the WWPP-irrigated soil were very close to those of the control soil. In this context, Sierra et al. (2001), demonstrated that the change in paste pH depends on the amount of organic matter oxidation.

Table 2 shows that EC values increased dramatically in the WWPP-irrigated soil, reflecting the high initial salinity of the effluent. Indeed, the EC of the WWPP-irrigated soil (3735 µm Scm^{-1}) exceeded the inhibitory value (estimated at 2000 µmS cm^{-1}) for sensitive crops (Semerjian 2011).

Soil organic matter (SOM) is a mixture of substances produced by living organisms and/or from the chemical decomposition of macromolecules (Annabi et al. 2007).The addition of the WWPP improved the SOM levels (Table 2), due to the initial contribution of exogenous residual organic matter (Mitra et al. 2007). However, the problem lies in the nature of this organic matter and its rate of biodegradability (Mekki et al. 2014). Microorganisms influence the structure and biological activity of a soil according to their types, their metabolism, and their synthetic products (Jastrow and Miller 1991).The TAM enumerated in the control soil (Table 3) was relatively low (25×10^6 CFU g^{-1} dry soil). This may be due to the soil's lack of organic matter and the region's dry climate (Mekki et al. 2013).

Addition of WWPP induced a small increase in the TAM count, to 75×10^6 CFU g^{-1} dry soil in the mixture

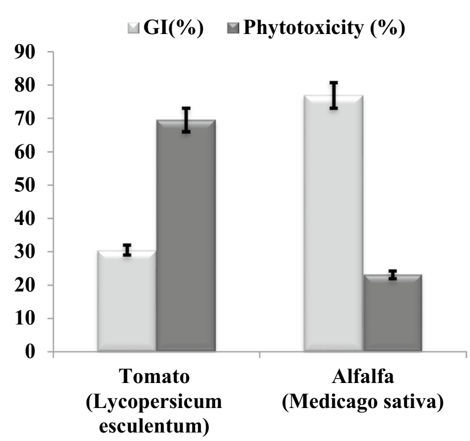

Fig. 2 Phytotoxicity of untreated wastewater from the phosphate ore processing (WWPP)

Table 2 Physicochemical and granulometric characteristics of soil irrigated with wastewater from phosphate ore processing (WWPP), in comparison with control soil (averaged values)

Characteristics	Control soil	WWPP soil
pH (25 °C)	7.9^a	7.91^a
EC (dS m^{-1}) (25 °C)	0.866^a	3.735^b
Dry matter (%)	93.05^a	91.57^a
Water content (%)	6.95^a	8.43^b
Organic matter (%)	2.04^a	3.15^b
Mineral matter (%)	91.05^a	88.41^b
Total nitrogen Kjeldahl (%)	0.12^a	0.58^b
Ammoniacal nitrogen (%)	0.03^a	0.18^b
TOC (%)	1.18^a	1.8^b
P (%)	0.05^a	0.23^b
Ca (%)	0.1^a	1.1^b
K (%)	1.02^a	4.2^b
Na (%)	0.08^a	2.3^b
Sand (%)	74.54^a	74.52^a
Clay (%)	16.26^a	16.33^a
Silt (%)	9.2^a	9.15^a

Standard deviation <5%; means followed within the same row by the same small letter are not statistically different

Table 3 Microbiological characteristics of soil irrigated with wastewater from phosphate ore processing (WWPP), in comparison with control soil (averaged values)

Microorganisms	Control soil	WWPP soil
TAM (10^6 CFU g^{-1} dry soil)	25	75
F (10^3 CFU g^{-1} dry soil)	28	46
SB (10^4 CFU g^{-1} dry soil)	2	12
TC (10^4 CFU g^{-1} dry soil)	ND	ND

TAM total aerobic microflora, *F* fungi; *SB* sporulant bacteria, *TC* total coliforms, *CFU* colony formant unit, *ND* not detected

WWPP/soil (Table 3). This increase in TAM could be explained by the mineral enrichment, which stimulated proliferation of the aerobic organisms (Mekki et al. 2015).

The presence of fungi was very weak in the control soil (28×10^3 CFU g^{-1} dry soil) and a non-significant increase was observed in the WWPP-irrigated soil (Table 3). This could be because fungi are more adapted to an acidic environment (Molope et al. 1987).

The number of sporulant bacteria (SB) in the control soil was about2×10^4 CFU g^{-1} dry soil. These bacteria were slightly stimulated in the WWPP-irrigated soil (12×10^4 CFU g^{-1} dry soil).Indeed, sporulation is a form of defense against inhibitors (Degens 1997). No coliforms were found in the control soil or in the WWPP-irrigated soil. This is good, since an abundance of TC is generally an indicator of contamination (Sridhara et al. 2008).

To evaluate the residual phytotoxicity of the WWPP-irrigated soil, tomato and alfalfa germination tests were carried out in extracts of soil irrigated with WWPP for 60 days. The GI percentages were compared with the extract of a control soil irrigated with pure water (Zucconi et al. 1981). The average GI for the tomato seeds was 66% compared to the untreated WWPP (30%), reflecting a phytotoxicity decrease from 70 to 34% (Fig. 3). The average GI (%) for the alfalfa seeds was 85% in the presence of the WWPP-irrigated soil extracts, reflecting a residual phytotoxicity of 15%. Such results confirm the role of soil as a "bioreactor" that contributes to the remediation and diminution of WWPP toxicity through its indigenous microflora (Rusan et al. 2015; Saeedi et al. 2010).

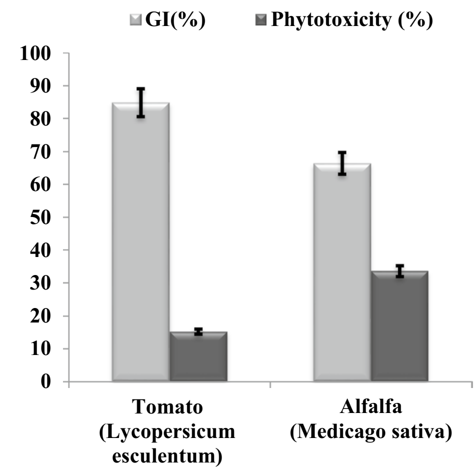

Fig. 3 Phytotoxicity of soil irrigated with wastewater from the phosphate ore processing (WWPP)

Conclusions

The objective of this work was to characterize wastewater from a WWPP plant and to study its potential toxicity. The WWPP had a very high turbidity of 609 NTU, a neutral or slightly alkaline pH, and a high salinity (an average EC of 9.64 dS m^{-1}). Averages values of COD and BOD_5 easily met wastewater discharge standards; however, the COD/BOD_5 ratio (4.34) was well above biodegradability requirements.

The WWPP contain far too much SM to meet the Tunisian standard for discharge of wastewaters. Much of the dry matter contained in this WWPP is in mineral form. Total nitrogen, residual phosphorus, and metals contents far exceeds the required standard for wastewater. This abundance of nitrogen and phosphorus presents a risk of soil contamination and waterway eutrophication.

Microbiological enumeration showed that WWPP was very poor in microflora. This is attributed to its high salinity and the dominance of minerals in this effluent. Regarding its potential toxicity, the WWPP had a very important BI of 76%. Its phytotoxicity ranged from 20 to 70% for alfalfa and tomato seeds, respectively. This phytotoxicity was reduced by adding this effluent to soil for 60 days. In fact, the residual phytotoxicity of the WWPP-irrigated soil is around was only 15 and 34%, respectively, for the tomato and alfalfa seeds.

Finally, this study has provided a clear idea of the physicochemical and microbiological characteristics as well as the potential toxicity of this effluent. Currently, this effluent is discharged into a sealed evaporation basin for evaporation, but this generates a lot of sludge and salts. In our future work, we will consider an appropriate treatment process for this wastewater.

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