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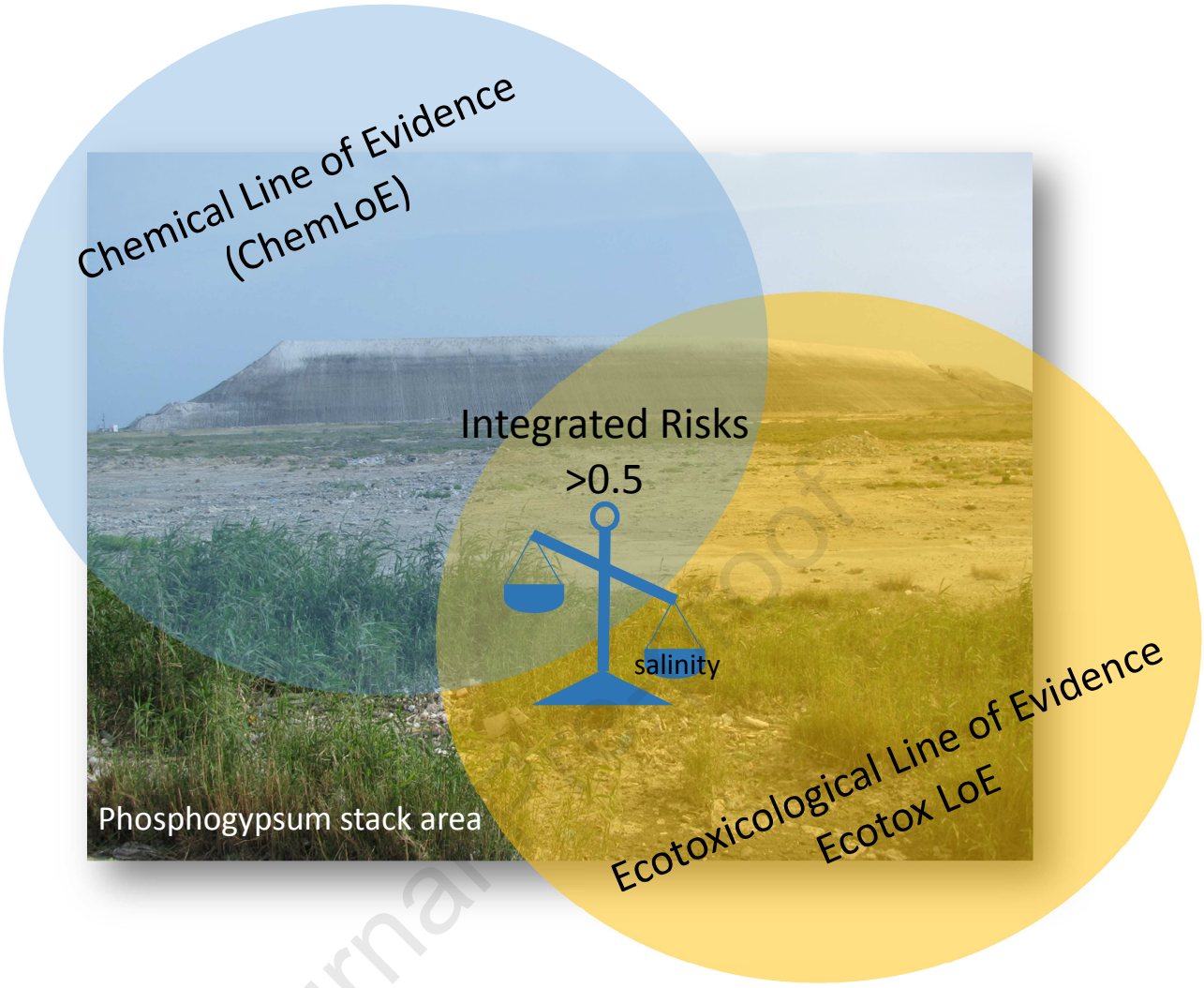

Application of a standard risk assessment scheme to a North Africa contaminated site (Sfax, Tunisia) -Tier 1

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Abstract

Phosphorus is a critical element to agriculture, consequently global phosphate rock demand will remain rising to feed a growing world population. The beneficiation of phosphorous ore gives rise to several tons of a waste by-product [phosphogypsum (PG)] which valorisation is limited, within other reasons, by the risks posed to environment and human health. Although threatening, the accumulation in stacks is the only procedure so far practiced by several countries as a means to get rid of this industrial externality. As part of a NATO Science for Peace Project (SfP 983311) this study describes the application of an environmental risk assessment (ERA) framework, to assess the risks posed by a PG stack to the surrounding soils, in Sfax, Republic of Tunisia. The ERA followed a weight of evidence approach, supported by two lines of evidence (LoE): the chemical (ChemLoE) and the ecotoxicological (EcotoxLoE). Integrated risks point for risk values greater than 0.5 in soils collected in PG stack surrounding area. Soil salinization, has likely contributed to the exacerbation of risks, as well as to the lack of consistency between both LoEs. This study highlights the need of rethinking the weight given to each LoE in ERA, in areas where soil salinization is a reality.

Keywords: msPAF, integrated risks, metals, lines of evidence, phosphogypsum

Capsule – Depending on possible confounding factors the weight of different lines of evidence that account for a risk assessment framework must be balanced.

1. Introduction

Concerns with contaminated sites within Europe started to be addressed in 1996, through the Concerted Action on Risk Assessment for Contaminated Sites (CARACAS) (Ferguson et al., 1998), later by the Soil Thematic Strategy (CEC, 2006) and by the Directive 2006/21/EC, of 15 of March, on the management of wastes from extractive industries (EC, 2006). However, and according to the World Mining Data report, from the Austrian Federal Minister of Economy, Family and Youth (Reichl et al., 2013), the exploitation of the mineral resources, at least in the last three decades, has been carried out in greater percentage (≥ 50% for ferro-alloy and non-ferrous metals) in developing countries, with a tendency for increasing since 2003. Despite that, environmental contamination is not a priority for these countries, and subsequently there is a deep lack of knowledge about the number of contaminated sites within their territories and the risks they represent for human health and even less for surrounding ecosystems. In an effort to start collecting data about hazardous waste sites, their dominant industrial pollutants, pathways of exposure and human populations at risk, the Blacksmith Institute set in motion the Toxic Sites Identification Program. Tunisia was one of the countries excluded from the Program by the lack of researchers with expertise for applying the Blacksmith Index, created by the modification of an original Hazard Ranking System (Ericson et al., 2013). Thus, and when Europe is discussing the application of existing risk assessment frameworks, like the one used in this study, to European countries that have not yet defined their own frameworks (Swartjes et al., 2008), it is also interesting to validate their application to developing countries and to empower local researchers for such purpose.

Tunisia is one of the world-leading countries in the extraction of phosphate rock and in the production of phosphoric acid and mineral fertilizers, which are two important national economic activities (http://www.gct.com.tn/). After fifty years of exporting all the extracted phosphate, Tunisia industry also started the transformation process by the wet acid method which gives rise to large amounts of a solid waste, named phosphogypsum (PG). This externality of the phosphoric acid production results from the following chemical reaction, through which fluorapatite is converted in gypsum, phosphoric and fluoride acids (Bisone et al., 2017; Rutherford et al., 1994):

$Ca_5(PO_4)_3F + 5H_2SO_4 + 10H_2O \longrightarrow 5CaSO_4(H_2O)_2 + 3H_3PO_4 + HF$

(fluorapatite, the mineral ore) (gypsum + phosphoric acid + hydrogen fluoride)

Mainly composed by calcium sulphate dehydrate (gypsum) with only 1% of phosphate, the PG has many impurities depending on the nature of the phosphate ore and/or the chemical treatment applied (Al-Hwaiti et al., 2010; Rutherford et al., 1994). Several radionuclides, especially those from the U-238 and Th-232 decay series (e.g. ^{238}U, ^{210}Pb, ^{210}Po, ^{226}Ra and its progeny), metals (e.g. Al, Cd, Cr, Cu, U, Sr, Zn), some rare earth elements (REE), sulphate ions and fluorides (F^-) are always present (Al-Masri et al., 2004; Bisone et al., 2017; Hammas-Nasri et al., 2016; Rutherford et al., 1996, 1994), being some of these elements of major concern to environment and human health. Given its richness in radionuclides PG was also recently classified as a naturally occurring radioactive material (NORM) (IAEA, 2013).

Generally, PG is produced in a proportion of about 5 tons per each ton of phosphoric acid (Papastefanou et al., 2006) and despite the suggestions of several potential applications for PG (Ajam et al., 2009; Kuryatnyk et al., 2008; Papastefanou et al., 2006; Saadaoui et al., 2017) including in agriculture, the valorisation of this waste has been greatly limited by its content in hazard elements. Consequently, in Tunisia and worldwide (e.g. Europe, Brazil, USA) only 15% of PG is recycled, while the other 85% is disposed on wet or dry stacks near the industrial plants (Da Conceição and Bonotto, 2006; Fuleihan, 2012; Pérez-Moreno et al., 2018; Tayibi et al., 2009), contaminating large land areas, frequently near the coast or main rivers (Corisco et al., 2017; Guerrero et al., 2019; Jalali et al., 2019). PG is currently being added to stacks at an annual maximum rate of 90 million t (not including the US) being forecasted that at these rates, the total amount of PG stored in stacks will attain 7–8 billion t by 2040 (IAEA, 2013). These stacks are usually perceived as a serious health hazard by local populations, mainly due to radon and dust emissions (Kuryatnyk et al., 2008; Wang et al., 2019). Studies demonstrated that PG may in fact pose a radiological threat to humans (Attallah et al., 2019). Further, PG stacks may also be an environmental hazards through leaching and runoff of contaminants into sediments, surface and groundwater resources, through wind drift of PG fine particles and subsequent deposition on neighbouring soils or nearby water resources (Ajam et

al., 2009; Guerrero et al., 2019; Kuryatnyk et al., 2008; Mosbahi et al., 2019; Tayibi et al., 2009), as well as through the erosion of PG stacks.

As part of a NATO Science for Peace project aiming to develop a new phytoremediation strategy to stabilize and rehabilitate a PG deposition area this study describes the first tier of a site-specific ecological risk assessment (ERA) process carried out in the surrounding area of two PG stacks in Sfax (Tunisia). This site-specific ERA was performed following the Dutch framework for the evaluation of contaminated sites (Jensen and Mesman, 2006), which in the meantime became an international standard (ISO, 2017). The methodology and rationale has already been successfully applied for the evaluation of risks posed by a metal contaminated area in the tropics (Niemeyer et al., 2010), as well as in firing ranges in Europe (Rodríguez-Seijo et al., 2017). It integrates information from three lines of evidence (LoE): the chemical (ChemLOE), the ecotoxicological (EcotoxLoE) and the ecological line of evidence (EcoLoE). The characterization of risks performed in tier 1, through a quantitative weight-of-evidence (WoE) approach, does support the decision either to finish the ERA, if the information available is considered sufficient and negligible risks are found, or to proceed collecting more information for the three LoE in a TRIAD approach assumed by this ERA framework.

The ERA process is particularly important when proposing remediation techniques (Moreno-Jiménez et al., 2011), since the risks posed to biological communities that are responsible for crucial soil services, have to be known if it is precisely intended to recover at least some of these services. Further, the ERA facilitates the planning and implementation of remediation works, as it could provide information about the vulnerable sub-areas, within a larger area, requiring a deeper intervention to mitigate the risks. In summary, it could be helpful in prioritizing sub-areas to be intervened based on their risk level, hence contributing to reducing operational costs.

In order to meet the objective described above, chemical data, namely pseudo total concentrations of metals in soils will be integrated with data from screening ecotoxicological assays both with: i) the whole soil matrix (solid phase test with the bacterial species *Allivibrio fischeri,* avoidance assays with the invertebrates *Folsomia candida and Eisenia andrei* and seed emergence assay with the plants *Lycopersicon esculentum* and *Avena sativa*, and ii) soil elutriates (growth inhibition assay with the green algae *Raphidocellis subcapitata* and mortality

assay with the cladoceran *Daphnia magna*). The selection of simple, standardized and low-cost assays for the lower tiers is a rule of thumb in site specific ERA processes (Critto et al., 2006; Rutgers and Mesman, 2011) and makes their application possible in different logistic and economic conditions.

2. Materials and Methods

2.1 Study site

Sfax is the second largest city of Tunisia in the Gulf of Gabes, located about 270 Km from Tunis. The phosphate industry is extremely well developed in this city (since more than sixty years), where the resulting PG has being dumped in two stacks: one is 12m high and covers an area of 40ha, and the other has a height of 30m and covers an area of 60ha (Wali et al., 2013). The area surrounding these stacks, located in the south of Sfax, was the site under evaluation in this study. According the Soil Atlas of Africa, the Sfax region is characterized by a high dominance of soils from the group of regosols (Jones et al., 2013).

2.2. Data collection for the Chemical LoE (ChemLoE)

2.2.1. Sampling design, soil collection and general physical and chemical characterization

Five transects were defined in the area surrounding one of the PG stacks (Figure 1). These transects, with four equidistant sampling points each, started near the stack and were directed outwards or positioned along the stack. A reference site (REF), located 9 Km northwest from the stack and without a recent soil use (based on Tunisian scientists knowledge of the area), was also selected. Hence, transect 1 was located between the PG stack and Sfax salt works; transect 2 was located between the PG stack and the municipal landfill; transects 3 and 4 started at the PG stack and were directed to the north, crossing the Oued El Maou (the drainage basin of an intermittent stream), and transect 5 was located between the stack and a metal recycling plant. A composite soil sample was taken from each site, in a total amount of 21 soil samples, of which two (T1.3 and T4.3) were rejected due to the extremely high water and silt contents, that prevented their processing in due time. Soil samples were brought to the laboratory, air-dried and sieved (4mm mesh size sieve for all ecotoxicological assays, except for

Microtox solid phase test for which a 2mm mesh size sieve was used as well as for physical and chemical parameters, for more details see section 2.3).

Soil pH was measured following the methodology proposed by the ISO 10390 protocol (ISO, 2005) in a soil:KCl 1M (1:5 m/v) suspension, using a 370 Jenway pH meter. Soil conductivity (mS cm^{-1}) and salinity were measured with a 470 Jenway conductivity meter in a soil:distilled water suspension (1:5 m/v) that was vigorously shaken for 1h and left to rest during a period of 12h (Dewis and Freitas, 1984; Soil and Plant Analysis Council, 2000). Soil organic matter was (OM %) measured by ignition of the dried soil samples, at 450^{o}C (Soil and Plant Analysis Council, 2000). Soil water holding capacity (WHC_{max} %) was determined according to the methodology described in the annex C of the guideline ISO 11268-2 (ISO, 2012a). All parameters were measured in triplicate for each soil sample. The particle size distribution (separation of the sand fraction), was made by mechanical analysis after oxidizing the organic matter content with hydrogen peroxide and dispersing the fine particulate matter with sodium hexametaphosphate (Sheldrick and Wang, 1993). The soil fractions of 2mm, 1mm, 500µm, 250µm, 150µm and 63µm were mechanically separated in a sieve shaker.

2.2.2. Pseudo-total metal contents analysis

For the analysis of total metal contents, 1g of each soil sample was wet digested with *aqua regia* (3 mL of HCl 37%, *pro analysis* PANREAC® and 1 mL of HNO_3, 65%, Suprapur Merck) in closed Teflon vessels. The vessels were placed in a sand bath at 60^{o}C, till complete dryness of the content of the flasks. Afterwards 10mL of HNO_3 4N were added to the flasks and the wet digested sample were then filtered through a 0.22 µm syringe filter, in order to eliminate remaining mineral particles. The filtrates were transferred to Falcon tubes of 25ml and the volume was adjusted with Milli-Q ultrapure water. The total content in Al, Cr, Fe, Ni, Cu, Zn, Cd, Pb and U were then measured in a Thermo-X-Series quadrupole ICP-MS (Thermo Scientific^{TM}), equipped with Ni cones and a Bugner nebuliser, and refrigerated by a Peltier System.

2.3. Data collection for the Ecotoxicological LoE (EcoToxLoE)

A set of screening assays recommended for the first tier of the risk assessment process (Jensen and Mesman, 2006) as described below, were carried out both with the whole soil

matrix and soil elutriates. As far as elutriates are considered, they were obtained by shaking a suspension of each soil in ASTM or Woods Hole MBL (hereinafter referred as MBL) media (1:4 m/v), for 12h, in an orbital shaker following the procedure adapted from (DIN, 1984). After this period, the suspensions were left to rest for 12h, the supernatant was decanted and stored at 4^{o}C, in the dark, for no more than a week, till being used in the assays with *Raphidocelis subcapitata* and *Daphnia magna*.

2.3.1. Algae growth inhibition assay

The microalgae *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen & O.M. Skulberg is reared in nonaxenic cultures, in flasks with MBL medium (Nichols, 1973) under semi-static conditions at 20±2^{o}C and continuous light exposure. Cultures were renewed every week, when the exponential growth phase is attained. The growth inhibition assay with *R. subcapitata* was performed according to a Growth Inhibition Test Protocol using a Freshwater Alga (Environment Canada, 2007) and adapted for 24-well microplates. For this purpose, 40 mL of the algal culture were transferred to a sterilized Erlenmeyer flask, from a log-phase culture (with 3 to 4 days). The number of cells mL-^{1} in the inoculum was determined through the counting of cells in a Neubauer chamber. Afterwards, the appropriate dilution of a microalgae inoculum was calculated to obtain an initial density of 10^4 cells mL^{-1} per plate well. For each soil elutriate, five dilutions (100, 50, 25, 12.5 and 6.25%; 3 replicates each) were tested by adding to each well 900 µL of the diluted elutriate plus 100 µL of the algae suspension. An additional well was filled only with 1000 µL of the respective elutriate dilution to correct the final absorbance due to the intrinsic colour of elutriates. In each microplate 3 wells were used as controls being each one filled with 900 µL of MBL medium plus 100 µL of the algae suspension. The plates were incubated for 72h under the same light and temperature conditions described for culture maintenance. After this period microalgae cells density was determined by measuring absorbance at 440nm, and by using the following equation previously obtained by the authors for the microalgae species:

$$Cells\ Density\ (ml\ L^{-1}) = -17107.5 + ABS_{440nm} X\ 7925350 \quad (r^2 = 0.98; p \leq 0.05)$$

One way ANOVA followed by a Dunnet test was performed to test for significant differences from the REF soil in average microalgae growth rate. The Levene's test was used to check for the homoscedasticity of variances.

2.3.2. Daphnia magna acute assays

Elutriates obtained from the different soil samples with ASTM medium were tested for their acute toxicity to *Daphnia magna* Straus clone K6, following the standard OECD guideline 207 (OECD, 2004a). For each soil elutriate the dilutions of 100, 50.0, 25.0, 12.5 and 6.25% were tested. Four replicates of 10 mL were prepared *per* dilution. Five neonates with less than 24h, obtained from the 3 to 5th broods of females from a culture reared in laboratory, were randomly assigned to each replicate. The replicates were maintained in the same laboratorial conditions of the culture (temperature: 20±1^{o}C; photoperiod: 16^{D}: 8^{L}) and checked after 24 and 48h of exposure. Immobilized neonates (organisms exhibiting no movement, for 15 seconds, after gentle prodding), were counted and removed from the vessels. No food was provided during the assay. Dissolved oxygen, pH and electrical conductivity were measured at the beginning the assay with a Wissenschaftlich Technische Werkstätten-WTW meter. Only the percentage of immobilized organisms in the non-diluted elutriate is provided, since no signs of immobilization were recorded for the neonates exposed to elutriate dilutions.

2.3.3. Microtox assay

To evaluate the toxicity of the whole soil sample to the bacteria *Allivibrio fischeri* (Beijerinck 1889) Urbanczyk et al. 2007, the Basic Solid Phase test protocol (consisting on testing nine dilutions of each soil sample suspension) was followed and its bioluminescence was monitored after 5, 15, and 30 minutes of exposure (AZUR, 1998). All tests were performed using the Microtox 500 Analyzer and the data was computed for each soil using the Software MicrotoxOmni Azur (AZUR, 1998). As it was not possible to determine ECx values, the highest percentage of bioluminescence inhibition recorded is presented.

2.3.4. Eisenia andrei and Folsomia candida avoidance assays

All test organisms were obtained from synchronized laboratory cultures maintained at constant conditions (temperature: 20±2ºC; photoperiod: 16h^{L}: 8h^{D}). The earthworms (*Eisenia andrei*

Bouché) were maintained in plastic boxes (with a volume of 10 to 50 L) containing a substrate composed by peat, dry and defaunated horse manure, water and $CaCO_3$ to adjust the pH between 6 and 7. The collembolans (*Folsomia candida* Willem) were maintained in plastic containers filled with a culture medium composed by moistened Plaster of Paris mixed with activated charcoal 8:1 (w:w). They were fed with granulated dry yeast twice a week. Avoidance assays followed standard ISO protocols namely ISO 17512-1 (ISO, 2008) and ISO 17512-2 (ISO, 2011) for *E. andrei* and *F. candida*, respectively. The tests were conducted in dual recipients containing the reference and test soils at opposite sides. For each tested soil 5 replicates containing 10 earthworms or 20 collembolans each were prepared. The organisms were added in the middle line of the containers after soil moisture had been adjusted to 40-45% of WHC_{max}. After 48h of incubation under the same conditions described for culture maintenance, the animals were counted at each side of the test containers and the avoidance percentage was calculated. Dual control chambers were prepared for both species, as controls, placing in both sides of the test containers the standard OECD soil (OECD, 2004b). A one-tailed Fischer exact test was used to test the null hypothesis of no significant avoidance of the test soils towards the REF, while a two-tailed test was used to test the hypothesis of no significant avoidance in the dual controls.

2.3.5. Seeds emergence assays with Lycopersicon esculentum and Avena sativa

Seeds emergence and growth tests with *Lycopersicon esculentum* Mill and *Avena sativa* L., one dicotyledonous and one monocotyledonous species, respectively were carried out according to the standard ISO 11269-2 and OECD protocols (ISO, 2012b; OECD, 2006). Only seed emergence data are reported in this study and used for the Tier 1 evaluation. Plant seeds were purchased from local suppliers. For each soil four replicates with $200g_{dw}$ of soil were prepared in plastic pots. A hole was made in the bottom of the pots to let a rope of cotton pass through it. The pots with soil were placed over other vessels containing distilled water. This water was absorbed by capillarity through the rope, keeping the soil always moistened. Water was continuously replenished in the vessels. At the beginning of the assay, a solution of nutrients (Substral® - fertilizer NPK: 6-3-6; nitrogen (N): 6%; phosphate (P_2O_5): 3%; potassium (K_2O): 6%; iron (Fe): 0.03%; trace elements: Cu, Mn, Mo and Zn) was provided to each pot diluted in

water according the recommendations of the supplier. Four control vessels, filled with OECD artificial soil (OECD, 2004b) was included. Twenty seeds were added to each replicate, for each plant assay. Pots were maintained at constant conditions of temperature (20 ± 2^{o}C), photoperiod (16hL: 8hD) and luminosity (25.000 lux). The test started after 50% of the seeds of the control soil have emerged, and lasted for more 14 days. The total number of seeds emerged in each replicate was counted. One way ANOVA followed by a Dunnet test was performed to test for significant differences from the REF soil in the average number of emerged seeds The Levene's test was used to check for the homoscedasticity of variances.

2.4. Risk calculation

In the first tier of ERA, the risks are calculated in two different steps: first for each LoE individually and then integrating all the LoEs. Hence, and regarding the ChemLoE, total concentrations of metals were used to calculate the multi substances (or elements) potentially affected fraction of species (msPAF) using the log-logistic concentration addition (CA) model (De Zwart and Posthuma, 2005; Lidman et al., 2016). This procedure was performed after correcting total concentrations of metals for background concentrations (based on the REF soil) and calculating the toxic pressure or the potentially affected fraction of species (PAF) for each metal individually according to following equations:

$$PAF_{1-n} = \frac{1}{(1 + (e^{\frac{log\mathrm{Hcp} - logTMC}{\beta}}))}$$

$$msPAF = 1 - ((1 - PAF_1)X(1 - PAF_2)X \ldots\ldots X(1 - PAF_n))$$

n – chemical substances taken into account in the risk estimation

HCp - Hazard concentration for a given percentage of species

TMC – pseudo-total metal concentration

β - constant value for each metal

From the list of metals analysed only Ni, Zn, Cd, Cr, Cu and Pb were used to calculate PAFs, since only for these ones HC_5 (hazard concentration that affects 5% of the species) are available (Jänsch et al., 2007). The β constants for each one of these metals were also given by Jänsch (personal communication). The HC_5 values from Jänsch et al. (Jänsch et al., 2007), were calculated based on the EC_{50} values for different species of animals, plants and microbial processes. It is assumed that no more than 5% of species or microbial processes will suffer an effect greater than 50% at this concentration level. These values were used in detriment of other soil screening values (SSVs) (e.g. the values from the Netherlands) because they do not require an adjustment for soil properties. Besides, no SSVs are available for Tunisia. SSVs are concentration thresholds above which certain legal actions are recommended or enforced, allowing as well screening out the sites for which the risks are too low to justify a more detailed evaluation (Ferguson et al., 1998; Provoost et al., 2008).

With respect to the EcoToxLoE the results obtained from the different ecotoxicological assays were scaled, following different strategies adjusted to the data generated by each assay. This scaling step is crucial, aiming to convert data from different assays into a unique effect scale, running from 0 (no effect) to 1 (maximum effect) (Jensen and Mesman, 2006; Rutgers and Mesman, 2011). Afterwards, the integration of risks of both LoEs was made, giving the same weight to different LoEs and, the standard deviation of the risks from individual LoEs was calculated.

3. Results and Discussion

3.1. Chemical Line of Evidence (ChemLoE)

Generally speaking, all soils surrounding the PG stack were neutral to basic (7.35±0.01 to 8.67±0.04) except the soils from the beginning of transect 1 and 3 (T1.1 and T3.1), which were slightly acidic (Table 1). These two soils were collected at two sites near the northwest limit of the stack, where they could be influenced by the spread of this waste, by erosion, runoffs or even management works (the irrigation of the pile with water for stabilization). The PG from Sfax has been characterized as highly acidic (pH between 2.9 and 4.26) (Ajam et al., 2009; Hentati et al., 2015), but our results are coincident with the work of (Jalali et al., 2019), who found some low soil pH values close to this PG stack. Regarding the conductivity, almost all the soils

showed extremely high conductivity values (> 1mS cm^{-1}), especially two soils from transects 4 and 5 which largely surpassed 10 mS/cm (Table 1). The exceptions were soils from transects 2 and 5 (T2.3, T2.4 and T5.4) which displayed very low conductivity values, even lower than the value recorded at the REF site (0.33±0.01 mS cm^{-1}). The high conductivity values occurred in parallel with high salinity values (Table 1). In fact, this stack was located near the coast and the salt marshes, hence high soil salinities were expected. Salinity promotes the dissolution of PG due to ionic strength effects and changes in the activity of ions in the saline solutions (Papanicolaou et al., 2009). The PG solubility usually occurs in parallel with the solubility and mobility of metals like uranium. This could in fact contribute to a high availability of metals in some of the soils with high salinity, despite the high pH values (Acosta et al., 2011).Eleven out of nineteen soils had a low organic matter content (< 2%) (Table 1). Only the REF soil and soils from transect 1, as well as site T3.1, had a medium organic matter content (between 2 and 6%) (Bodenkunde, 1982). As far as the soil texture was considered, some of the soils displayed a low percentage of the fraction < 63µm (<16.5%), namely soils T2.4, T4.2 and T4.4, what is also translated in a lower percentage of clay, concomitant with a low organic matter content (Table 1). Both factors have been described as determinant in the fixation of metals in the soils, reducing their bioavailability, even in saline soils (e.g. (Bartkowiak, 2017; Peijnenburg et al., 2012)).

Table 2 displays the total metal contents recorded in all the soil samples analysed in Sfax study area, as well as the HC_5 (*see Risks calculation section*) values provided by Jänsch et al. (Jänsch et al., 2007) and the Environmental Health Canadian Soil Quality Guideline Values (EH-SQGV) available (CCME, 2015, 2007, 1999a, 1999b, 1999c, 1999d, 1997). While the former values were used for the calculation of risks of the *ChemLoE*, the Canadian values were used only for comparative purposes. Comparatively to the other soils, the REF soil had the highest Fe and Ni total contents. However, all the metals analysed were below the corresponding EH-CSQG, and only total Cr surpassed the HC_5 value proposed by Jänsch et al. (Jänsch et al., 2007). All the soils from transect 1 had Cd levels above the corresponding HC_5 value and/or the EH-CSQG values (CCME, 2015, 2007, 1999a, 1999b, 1999c, 1999d, 1997). Total Cr content also surpassed the HC_5 value in the soil T1.1. No metal exceeded the soil quality values for all the soils from transect 2. Several soils from transects 3, 4 and 5 also

surpassed the HC_5 value for total Cr (Jänsch et al., 2007). The same was observed for Cu in the soil T4.4 and T5.1, which surpassed both soil quality guideline values and for Zn at soil T5.1. Adding up the total contents of all the metals, soils from transect 1 (T1.1, T1.2 and T 1.4), T4.1, T4.4, T5.1 and T5.2 had the highest total content of metals.

3.2. Ecotoxicological Line of Evidence (EcotoxLoE)

All the assays performed complied with validity criteria set by the corresponding protocols. All elutriates tested displayed dissolved oxygen levels well above the hypoxia level of 3mg/L (Table S1, supplementary material). Table 3 displays toxicity data obtained for all the assays both with the whole soil matrix and with soil elutriates. Only data for non-diluted soils and elutriates are reported and were used for risk calculations. In a general way, the soils were more toxic that the corresponding elutriates, which is not a surprise considering that elutriates only contain the fraction of chemically available and bioavailable contaminants, thus providing information about the soil retention function. However, the results were not coincident, i.e. the soils causing serious toxic effects on some species, had less effect on other species and *vice versa*, suggesting that different elements (metals, salts or both) of these complex matrices (both soil and elutriates) are affecting test organisms. Five soil elutriates had an acute effect on *D. magna* causing 100.0±0.0% of immobilization. Soil metal contents could explain these results, at least for elutriates from soils T1.4, T5.2 and T5.3. In the other soils, different factors must have been responsible for such acute effects. Salt is also a stress agent in these soils and could have been the cause of *D. magna* immobilization, at least in some soils (e.g. T4.2). Schuytema et al. (1997) observed percentages of mortality higher than 50% for *D. magna* exposed for 7 days to saltwater with conductivity values equal or higher than 11.3 mS cm^{-1}. A similar conductivity value was recorded for T4.2 soil, and an even higher value was recorded for the soil's elutriate (15.9 mS cm^{-1}) prepared with ASTM hard water medium (Table S1). But no immobilization was recorded for soil T4.4 which elutriate displayed a conductivity value 1.89 times lower (Table S1). The shortest exposure period, compared to the previous described study, may have also contributed to the lowest toxicity. Nevertheless, it is important to highlight that different types of salts may be present in this area (sea salts and PG salts), with different toxicity for cladocerans, as shown by Mount et al. (2016). These differences in salts (in terms of origin) may also have

contributed to the variability in the toxicity of soil elutriates even for those from soils with high conductivities. The low OM% of some other soils (e.g. T5.3) may have contributed to a higher bioavailability of metals, other than those considered for risk calculation (e.g. Al) or other contaminants not analysed in this study (e.g. radionuclides, organic contaminants). Gostomski. (1990) reported an LC_{50} value for Al >25.5 mg L^{-1}, for *D. magna*, at a pH=7.61. Since the Al concentrations in the soils were very high it is highly possible that at least in some soil elutriates, higher Al concentrations have been attained. Furthermore, the PG from Sfax has been reported as highly enriched with Sr. Jalali et al. (2019) reported a Sr average concentration of about 698±370 mg kg^{-1}. Although metal contents of soil elutriates were not analysed, with such a high concentration of Sr in PG, acute toxic values to daphnids (LC_{50}-48h for *Daphnia hyalina* 75 mg L^{-1}) may have been attained in the elutriates (Baudouin and Scoppa, 1974). Zmemla et al. (2016) included Sr as well as Zn in the group of metals of PG with the highest mobility. And in fact, Hentati et al. (2015) showed the high solubility in water of Zn and C, and subsequently a high concentration of these elements in elutriates obtained from soil mixed with different percentages of Sfax PG. The same rationale could be applied to other elements as fluoride, based on the LC_{50} values (Shamsollahi et al., 2015). It has indeed been proven that salts may also increase the availability of metals in soils, and subsequently their mobility to soil elutriates (Acosta et al., 2011).

R. subcapitata was less sensitive than *D. magna* to soil elutriates. Significant percentages of microalgae growth inhibition were recorded only for soils T1.4, T4.4 and T5.2. Metals may have been once again the main factors responsible for inhibiting the algae growth, since all these soils had metals above soil quality guideline values and were part of the group of soils with the highest content of metals. The acidic pH of the soil T1.4's elutriate, may have also contributed for an enhanced bioavailability of metals or for complex interactions between protons and contaminants (Table S1). Soil T5.2 also had the highest content of Al recorded for Sfax soils (19.6 g kg^{-1} $soil_{dw}$). Gostomski (Gostomski, 1990) reported two EC_{50} values for Al and for *Selenastrum capricornutum* (now *R. subcapitata*) biomass production, of 0.57mg L^{-1} (pH=7.6) and 0.46 mg L^{-1} (pH=8.2). Similar concentrations were likely attained in some elutriates, like the one from soil T5.2. *Pseudokirchneriella subcapitata* (now *R. subcapitata*) is also sensitive to salts toxicity (Simmons, 2012). In this previous study microalgae species also

showed different sensitivities to different salts according to, the following ascending order: KCl=NaCl>Na_2SO_4=$CaCl_2$>K_2SO_4, with corresponding EC_{50} values ranging from 1.7 mS cm^{-1} (KCl) to 5.8 mS cm^{-1} (K_2SO_4) for cells density. Hence, it is likely that at least for soils T4.4 and T5.2, with the highest conductivity values, and in particular for the soil T5.2's elutriate (conductivity 11mS cm^{-1}) (Table S1) salts also had a role in algae growth inhibition.

As regards the assays with the whole soil matrix, but also for aquatic species, the bacterium *A. fischeri* was the less sensitive species to Sfax soils. The Microtox® assay is not sensitive to salinity, because *A. fischeri* is a marine species, and it has been shown that salinities greater than that promoted by the osmotic adjustment of the bacteria test medium, may stimulate the luminescence masking the toxicity of metals (Cook et al., 2000). This soil had a high concentration of total Cr, well above the HC_5 value presented by Jänsch et al. (2007), for this metal. Nevertheless, different authors reported the lack of sensitivity of this assay to Cr (VI) (Codina et al., 1993; Fulladosa et al., 2005). It is also known that iron is a strong reducer of Cr (VI) (Shettlemore and Bundy, 2001), giving rise to Cr(III), which may have induced a different response on *A. fischeri*. The same authors, also argue that Cl^- ions may enhance the toxicity of metals through potentiating mechanisms. This could be an explanation for the percentage of bioluminescence inhibition recorded for soil T1.1 (30%), where a high salinity value co-occurred with a Cd content above soil quality guideline values, despite the low sensitivity of *A. fischeri* also reported for Cd (Codina et al., 1993). But contradicting these explanations, the soil T5.1, with high total metal concentrations, including Cu and Zn well above soil quality guideline values, and an intermediate salinity did not induce any response in *A. fischeri,* despite the great ability of Cu and Zn for inducing toxic effect on the bacteria, when compared with Cr (VI) (Fulladosa et al., 2005). In a general way, and based on PG chemical nature, Ca^{2+} ions, which are at high concentrations at these soils surrounding the stack and with a great mobility to soil elutriates (Hentati et al., 2015), may have contributed for reducing the toxicity of metals, like Cd as shown by Bessa et al. (2017). The low sensitivity of *A. fischeri* to metals has already been recorded by other authors questioning the recommendations for using this test as a screening tool in risk assessment procedures, for discriminating environmental samples, as it can lead to false negatives (Teodorovic et al., 2009). However, the same authors ultimately agree that the *A. fischeri* assay should be part of a vast battery of assays as they can identify other toxic

substances that are not under evaluation. The *Arthrobacter globiformis* contact test may be a good alternative for the risk assessment of areas with salinized soils, although the sensitivity of this test to soil salinization may exacerbate and mask the effects of other soil contaminants. Marques et al. (2014) applied this test to make a first screening of the soils evaluated in this study and recorded a dehydrogenase activity inhibition percentage (DAI%) greater than 45%, for all the soils from transect 1 and 3, as well as for T2.1, T4.2, T4.4, T5.2 and T5.3 soils. A positive correlation was found only between DAI% and soil salinity or conductivity.

Clearly and as previously mentioned, the soils surrounding the PG stack area were highly toxic to the invertebrate species. These results are thus a clear demonstration of the extremely complexity of the soils under evaluation. As regards to plants, the monocotyledonous species *A. sativa* was even more sensitive, presenting the lowest percentages of seeds germination, comparatively to the dicotyledonous species (Table 3). A no significant inhibition ($p>0.05$), comparatively to the REF soil, was recorded only for soils T2.3 for both species and T2.4 for *A. sativa.* (Table 3). In fact, these soils were part of the group of soils with lower total metal contents, and also had the lowest conductivity and salinity values (Table 1 and 2). Seeds germination data also displayed a high variability between replicates, despite the careful soil homogenization, before testing and, the obvious concern in guarantying similar light and moisture conditions in all the pots. Thus, these results are another evidence of the complexity of the soils under evaluation, which is likely affecting the balance in plants uptake of ionic species.

Soil invertebrates, both collembolans and oligochaetes, have clearly avoided most of the soils, except those from transect 2 (T2.1, T2.3 and T2.4) as well as T5.4. Instead of being avoided, almost all these latter soils were preferred by these invertebrates. These soils were the ones with the lowest salinity and conductivity values, lowest metal contents and the soil T5.4 was also one of those with the highest organic matter content. Further, in a general way, *E. andrei* displayed highest avoidance percentages than *F. candida,* and once again this happened not only for soils with high concentrations of metals, but also for soils from transect 3, which displayed salinities above 3. Owojori and Reinecke. (2009) reported an EC_{50} for *E. fetida* avoidance of a natural saline soil of 0.56 (95% confidence limits: 0.44-0.71) dS m^{-1}. This can explain the preference rather than the avoidance of soil T2.2, T2.3, T2.4 and T5.4, which displayed conductivities below this level, but not the preference of soil T2.2. However, in

another study, the same authors also proved that electric conductivity cannot be used to correctly predict the toxicity of salts to *E. fetida*, as there is an ion-dependent toxicity to earthworms (Owojori and Reinecke, 2014). For the other soils, the clear avoidance behaviour of invertebrates, was likely once again caused by complex interactions between salts and metals. The highest sensitivity of *E. andrei,* recorded in this study when compared to *F. candida*, is also coincident with observations of Owojori et al. (2009). These authors concluded that for the four species tested, *E. fetida* was the most sensitive to soil salinity and *F. candida* was the less sensitive one. Pereira et al. (2015) also recorded a lower sensitivity of *F. candida* when exposed to OECD artificial soil moisturized with a NaCl solution, comparatively to potworms (*Enchytraeus crypticus*), but precisely the opposite when the soil was moisturized with seawater, demonstrating once again that there are a salt specific toxicity, which also differs between species. The exoskeleton of springtails and its ability to offer a higher protection against external stressors is likely, once again, the main explanation for the differences in sensitivity recorded in this study, as well as in other studies, between both invertebrate species.

Risks calculation

The low total metal content of soils was responsible by the lower risk values estimated based on the Chemical LoE (Table 4) for all the soils from transects 2 and 3, as well as for soils T4.2, T5.3 and T5.4 located far from the stack. All soils with risks higher than 0.5, corresponded to the soils with the highest total metal concentrations (considering all the metals except Al and Fe) (Table 2). This was not a surprise, as the risks are calculated by applying a response addition model to the toxic pressures obtained for the different metals under analysis (De Zwart and Posthuma, 2005). The highest values of chemical risks (≥ 0.75) were recorded for soils T1.2, T4.4 and T5.1. All the soils with risk values higher than 0.5 for the ChemLoE also had high risk values based on the EcotoxLoE (ranging between 0.79 and 0.92), suggesting that metal contamination from the PG stack and also from the metal recycling plants are likely responsible for the estimated risks at these points. Furthermore, the results of the ChemLoE suggest that local soil contamination seem to be confined to short distances from the stack. Wind transport to greater distances than those tested cannot be discarded. However, it is possible that PG

material is well confined to the stack, due to the high atmospheric humidity of the coastal area, as well as through the continuous moistening of the stack that is made by the company and that may hinder the dispersion of PG by wind. Thus, the pore water of piles that emerges on the edges is likely also responsible by the spatial limited dispersion of PG contaminants, in the Sfax study area. Given the high volumes produced and due to its chemical characteristics the pore water has being pointed out as the main route of dispersion of pollutants (Pérez-López et al., 2018, 2015). In fact, and despite the aridity of the area, the water content of the soil samples collected was so high that in at least two cases (T1.3 and T4.3) it was not possible to dry the field samples during the time the international project team stayed in Sfax. Such high water content can result from the up-rise of coastal aquifers as well as from leaking out of the PG stack.

High integrated risk levels (above 0.5) were recorded for soils from transect 1, 3 (except T3.2), transect 4, and transect 5 (except T5.4), however the standard deviation of this risk, in some of the soils was above 0.4, as in these cases it was mainly the EcotoxLoE that accounted for the integrated risks (table 4). Salinity seems to be the main responsible for the lack of consistency between both lines of evidence. Chelinho et al. (submitted) by performing long-term toxicity tests with soil invertebrates, more appropriate for a Tier 2 of the ERA process, confirmed that these soils seriously compromised the reproduction of *F. candida*, *Enchytraeus bigeminus* and *Hypoapsis aculeifer,* and once again pointed for soil salinity, as the main factor responsible for the effects recorded. In a general way these results were also coincident with those recorded by Marques et al. (2014). However, by looking again at the main soil properties, it is possible to realise that salinity cannot be the only factor responsible for the observed effects and for the lack of coherence between both lines of evidence. As above described other metals not analysed in this work (e.g. Sr and F), salinity, radionuclides, as well as organics released from the landfill and the mixture of all these contaminants may be responsible by the toxicity of soils surrounding the Sfax PG stack. Pérez-Moreno et al. (2018) demonstrated that in a PG stack from Huelva (Spain), a meaningful proportion of at least radionuclides, such as ^{210}Po and ^{238}U, may have a high mobility. Further, another aspect that was highlighted by Hentati et al. (2015)(Hentati et al., 2015)(Hentati et al., 2015)(Hentati et al., 2015)(Hentati et al., 2015) and that needs to be addressed is the role of the high concentrations of calcium associated with

soils affected by PG, both in the uptake of metals, as well as in the disturbance of the metabolism of the organisms, which may have an opposite role regarding the response of soil invertebrates to the soils affected by PG. Calcium in excess may compete with other cations reducing its uptake by soil invertebrates (Ardestani and Van Gestel, 2013), but may also rapidly affect the cellular metabolism of organisms. Exposure of *E. andrei* to metals and radionuclides has proved to upregulate genes involved in the activation of Ca^{2+} metabolism and homeostasis mechanisms involved in the protection of cells from massive Ca influx (Lourenço et al., 2013).

Conclusion

The application of the first tier of a standardized risk assessment process, supported by only two out of three lines of evidence (without the Ecological Line of Evidence) was able to characterize the risks posed to soil biota, in the surrounding area of the PG stack, located near the coast in the city of Sfax (Tunisia). The complexity of soil contamination patterns in this area, involving both different types of salts, metals and radionuclides, increased the power of the EcotoxLoE in the identification of the hazard of individual soil samples. Such complexity in soil contamination, especially in this area, and in other areas similar to this one, where various sources of pollutants are present, could be a good reason to give a highest weight to the EcotoxLoE in the risk assessment process. However, it is important to take into account that, depending on the intended remediation strategies, as well as on the future use of the area, the EcotoxLoE may be also responsible for an overestimation of the risks, when soil salinization is involved. In these cases, rather than performing more ecotoxicological assays, a more detailed characterization of soil contamination in tier 2, may be required to confirm the disagreement between both LoE. Tunisia, in particular, has other three PG stacks across the country, therefore, this study could be considered as an example to be extended for the assessment and management of those contaminated zones.

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Table 1. Geographical coordinates and general physical and chemical characterization of soil samples collected in the PG stack area in Sfax, Tunisia. V Averages and corresponding standard deviations (average ± STDEV).

Samples	Geographical coordinates	pH_{KCl}	Conductivity (mS. cm^{-1})	OM (%)	WHC_{max} (%)	Salinity	Silt+clay (%)
REF	34º43’49,64’’N – 10º38’21,45’’E	7.81±0.02	0.33±0.01	4.36±0.38	60.5±0.8	0.0±0.0	46.3
T1.1	34º42’05,39’’N – 10º44’07,55’’E	6.23±0.04	8.40±0.01	3.79±0.60	75.9±5.0	5.2±0.0	30.1
T1.2	34º42’02,07’’N – 10º44’07,21’’E	8.16±0.08	6.77±0.03	3.50±1.50	84.6±5.2	4.1±0.0	32.4
T1.4	34º41’50,73’’N – 10º44’08,08’’E	8.29±0.11	6.20±1.57	6.49±0.44	52.4±11.7	5.3±0.0	26.9
T2.1	34º41’26,84’’N – 10º44’03,25’’E	7.85±0.07	3.18±0.09	1.52±0.31	43.0±1.7	1.8±0.1	30.3
T2.2	34º41’28,47’’N – 10º44’00,33’’E	7.78±0.06	2.03±0.04	0.84±0.19	21.1±0.4	0.67±0.04	27.6
T2.3	34º41’29,77’’N – 10º43’57,57’’E	8.06±0.03	0.14±0.01	1.40±0.50	32.7±2.2	0.63±0.01	22.5
T2.4	34º41’31,11’’N – 10º43’54,84’’E	7.99±0.11	0.18±0.00	0.50±0.20	37.6±4.2	0.83±0.00	16.5
T3.1	34º42’08,09’’N – 10º44’08,08’’E	6.56±0.01	8.69±0.40	4.25±0.25	50.8±3.6	5.4±1.1	26.0
T3.2	34º42’11,13’’N – 10º44’08,56’’E	8.67±0.04	7.00±1.98	1.50±0.25	39.8±3.9	6.0±2.0	20.1
T3.3	34º42’13,96’’N – 10º44’08,88’’E	8.50±0.03	5.72±0.30	0.64±0.34	38.2±3.4	3.4±0.3	23.1
T3.4	34º42’16,88’’N – 10º44’09,51’’E	8.37±0.14	6.38±0.06	1.79±0.09	59.1±5.9	3.9±0.1	27.1
T4.1	34º42’14,09’’N – 10º43’45,31’’E	8.05±0.03	2.40±0.03	2.46±0.25	35.8±0.9	1.2±0.0	28.6
T4.2	34º42’16,04’’N – 10º43’47,83’’E	8.31±0.01	11.8±0.5	2.42±0.31	29.9±0.5	7.5±0.5	14.8
T4.4	34º42’20,88’’N – 10º43’50,41’’E	8.30±0.01	23.4±0.4	1.06±0.03	28.8±0.9	29.5±0.0	12.6
T5.1	34º41’31,46’’N – 10º43’50,30’’E	7.35±0.01	2.78±0.02	1.12±0.10	42.8±1.3	1.5±0.0	21.4
T5.2	34º41’28,45’’N – 10º43’48,20’’E	8.35±0.01	17.6±0.4	1.49±0.19	154±14.5	11.6±0.1	70.4
T5.3	34º41’26,52’’N – 10º43’48,16’’E	8.43±0.03	6.75±0.96	0.66±0.03	39.6±3.4	4.1±01.0	28.9
T5.4	34º41’24,32’’N – 10º43’47,22’’E	8.07±0.04	0.14±0.03	3.31±0.05	47.3±0.3	0.49±0.03	22.3

OM%-organic matter percentage; WHCmax (%) – maximum water holding capacity in percentage.

Table 2. Total content of metals recorded in soils from Sfax's study area (mg kg^{-1} $soil_{dw}$ except for Al and Fe which are in g kg^{-1} $soil_{dw}$)

	Al	Cr	Fe	Ni	Cu	Zn	Cd	Pb	U	Total*
REF	2.93	**40.0**	17.4	15.7	8.5	51.0	0.2	9.6	0.9	126
T1.1	6.83	20.3	3.38	2.7	3.1	66.3	6.6	5.1	3.9	**108**
T1.2	4.30	9.2	1.84	4.2	3.5	82.8	**10.9**	3.9	1.6	**116**
T1.4	5.60	10.7	3.00	5.5	4.6	72.5	**6.6**	4.6	1.6	**106**
T2.1	11.1	15.7	6.03	6.1	5.8	26.0	0.3	6.9	0.7	61.6
T2.2	8.98	13.1	5.00	5.1	3.6	18.4	0.1	4.5	0.4	45.1
T2.3	7.75	12.4	4.35	4.6	6.0	23.7	0.1	5.8	0.4	52.8
T2.4	6.43	9.8	3.88	3.5	2.7	17.0	0.1	4.0	0.3	37.3
T3.1	8.53	13.6	6.88	4.9	3.5	16.7	0.1	4.5	0.3	43.6
T3.2	7.93	16.3	4.55	18.3	8.8	32.0	1.7	6.5	1.6	85.2
T3.3	5.60	3.4	0.41	3.9	13.3	19.8	0.2	11.9	1.4	53.9
T3.4	4.00	7.0	3.33	3.6	36.5	23.5	0.3	23.8	0.6	95.2
T4.1	9.73	60.3	8.18	9.6	31.5	137	1.0	28.0	1.2	**268**
T4.2	4.95	8.8	2.55	2.8	2.9	13.9	0.2	5.0	0.9	34.4
T4.4	5.18	11.4	4.35	8.4	133	93.0	0.2	32.8	1.0	**279**
T5.1	3.35	28.0	4.55	12.4	**137**	**283**	3.0	144	3.1	**610**
T5.2	19.6	35.0	9.80	10.7	21.7	127	1.2	25.0	8.4	**229**
T5.3	6.93	10.7	4.05	4.5	5.3	23.9	0.1	4.6	0.8	49.9
T5.4	8.35	14.0	4.43	4.7	5.7	36.8	0.5	8.5	1.3	71.4

HC5	NA	5.0	NA	64.0	55.0	160.3	6.8	163.5	NA
EH-CSQG	NA	64.0	NA	50.0	63.0	200.0	3.8	70.0	33.0

HC5 - Hazard concentration for 5% of the species based on EC50 values (Jänsch et al., 2007).

EH-CSQG (Environmental Health - Canadian Soil Quality Guidelines): CCME (1997,1999a-d, 2007, 2015).

*Sum of total metal contents excluding iron and aluminium.

NA - not available; bold letters highlight concentrations surpassing soil quality values and the highest total values.

Table 3. Ecotoxicological data recorded for the different soils collected in the PG stack surrounding area (Sfax, Tunisia).

	A. fischeri	*E. andrei*	*F.candida*	*L. esculentum*	*A. sativa*	*R. subcapitata*	*D. magna*
	% biolumin inhibition	% avoidance ± SD	% avoidance ± SD	Seeds emergence (%)	Seeds emergence (%)	% growth inhibition[c]	% mortality ± SD
Ref	42	---	---	91.3 ± 11.1	95.0 ± 5.0	7.5	0
T1.1	10	95.6 ±9.9*	92.0 ± 8.4*	18.8 ± 17.9 [a]	0.0 ± 0.0 [a]	0.0	0
T1.2	30	100.0 ± 0.0*	79.1 ± 24.3*	13.3 ± 10.40 [a]	0.0 ± 0.0 [a]	0.0	0
T1.4	NT	70.7 ± 35.9*	79.6 ± 23.9*	36.6 ± 333[a]	15.0 ± 23.8 [a]	69.1[b]	100.0 ± 0.0
T2.1	NT	28.8 ± 51.3	40.3 ± 17.3*	27.5 ± 8.6 [a]	3.75 ± 2.5 [a]	0.0	0
T2.2	--	-94.0 ± 13.4	6.4 ± 19.5	53.8 ± 27.2 [a]	16.7 ± 20.2 [a]	--	--
T2.3	NT	-64.0 ±27.0	-60.4 ± 23.1	88.8 ± 11.1	78.3 ± 20.2	14	5
T2.4	NT	-54.0 ± 21.9	-48.6 ± 14.9	80.0± 0.0	25.0 ± 15.0 [a]	---	0
T3.1	15	93.3 ± 11.5*	77.4 ± 31.3*	7.5 ± 3.5 [a]	0.0 ± 0.0 [a]	0.0	0
T3.2	NT	60.0 ± 34.7*	56.5 ± 34.3*	45.9 ± 28.0 [a]	6.7 ± 7.6 [a]	0.0	1
T3.3	28	96.7 ± 5.8*	70.0 ± 20.5*	---	0.0 ± 0.0 [a]	0.0	0
T3.4	NT	100.0 ± 0.0*	74.5 ± 27.9*	35.0 ± 14.4 [a]	0.0 ± 0.0 [a]	0.0	0
T4.1	NT	53.8 ± 25.7*	66.4 ± 31.5*	0.0 ± 0.0 [a]	1.7 ± 2.9 [a]	0.0	0
T4.2	NT	100.0 ± 0.0*	73.0 ± 27.4*	58.8 ± 17.0 [a]	48.3 ± 28.4 [a]	0.0	100.0 ± 0.0
T4.4	NT	100.0 ± 0.0*	69.4 ± 31.5*	8.3 ± 2.9 [a]	1.25 ± 2.5 [a]	56.7 [b]	0
T5.1	NT	98.0 ± 4.5*	55.4 ± 27.4*	25.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0	0
T5.2	10	100.0 ± 0.0	59.6 ± 46.5*	30.0 ± 21.8 [a]	1.7 ± 2.9 [a]	64.2 [b]	100.0 ± 0.0
T5.3	NT	97.8 ± 5.0	100 ± 0.0*	35.0 ± 21.1 [a]	6.7 ± 7.6 [a]	0.0	100.0 ± 0.0
T5.4	NT	-84.0 ± 26.1	-56.3 ± 17.6	21.5 ±11.08 [a]	25 ± 8.7 [a]	0.0	100.0 ± 0.0

***** Significant avoidance percentage ($p<0.01$) according to the Fischer exact test; a- significant differences from the REF soil (Dunnet test: $p<0.05$); b- significant differences from the REF soil in microalgae growth rate (Dunnet test: $p<0.05$); c- percentage of growth inhibition towards the CTL with MBL medium.

Table 4. Risks estimated for the soils collected in the PG stack surrounding area (Sfax, Tunisia) for the Chemical LoE, the EcotoxL Loe, Integrated risks (IR) and corresponding standard deviation (SD).

	REF	**T1.1**	**T1.2**	**T1.4**	**T2.1**	**T2.2**	**T2.3**	**T2.4**	**T3.1**	**T3.2**	**T3.3**	**T3.4**	**T4.1**	**T4.2**	**T4.4**	**T5.1**	**T5.2**	**T5.3**	**T5.4**
Chem LoE	0.0	**0.51**	**0.65**	**0.53**	0.02	0.0	0.0	0.0	0.0	0.21	0.09	0.37	**0.58**	0.0	**0.75**	**0.93**	**0.51**	0.0	0.04
Ecotox LoE	0.0	**0.79**	**0.84**	**0.80**	**0.55**	0.31	0.04	0.22	**0.80**	**0.51**	**0.88**	**0.82**	**0.79**	**0.82**	**0.87**	**0.81**	**0.92**	**0.87**	**0.71**
IR	0.0	**0.68**	**0.77**	**0.69**	0.34	0.17	0.02	0.12	**0.56**	0.38	**0.67**	**0.66**	**0.70**	**0.58**	**0.82**	**0.89**	**0.80**	**0.63**	0.47
SD of IR	0.0	0.20	0.14	0.19	0.37	0.22	0.03	0.15	0.57	0.21	0.55	0.32	0.15	0.58	0.09	0.09	0.29	0.61	0.47
SD * 1.73	0.0	0.34	0.24	0.34	**0.64**	0.38	0.05	0.27	**0.98**	0.36	**0.96**	**0.55**	0.26	**1.00**	0.15	0.16	**0.50**	**1.06**	**0.82**

Bold values stand for risk values higher than 0.5 and SD values higher than 0.4.

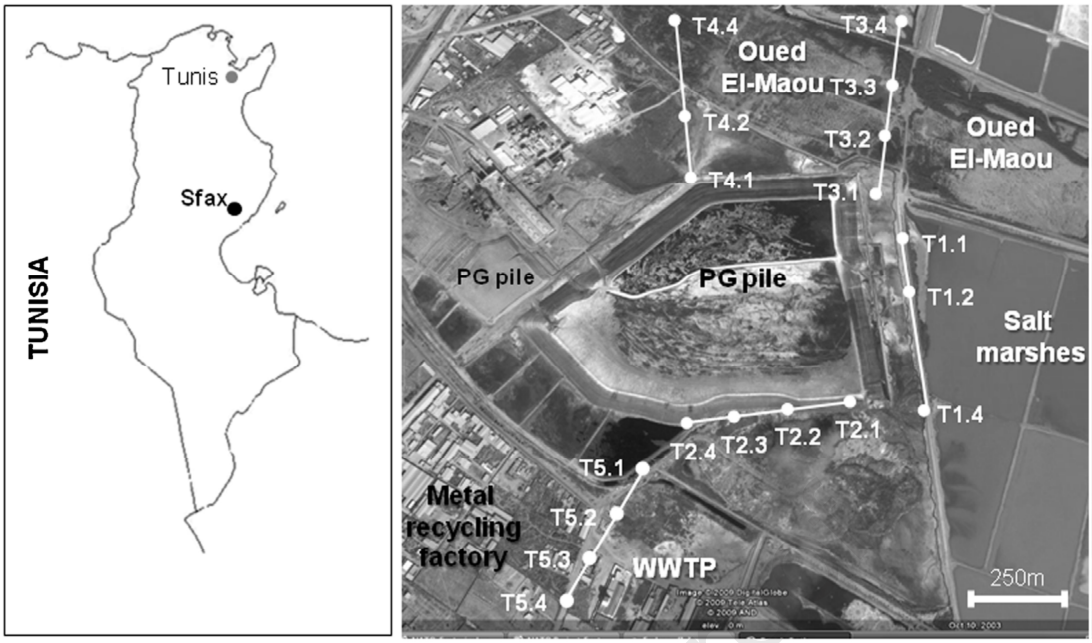

Figure 1. Google Earth aereal image of the study site in Sfax Tunisia. Soil transects and corresponding sampling spots are illustrated (Marques et al, 2014).

Highlights

- Tier 1 of risk assessment framework may include only two lines of evidence (LoE)
- Soil salinity accounted for the lack of coherence between LoE
- Different weights given to each LoE may overcome the confounding effect of salinity

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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