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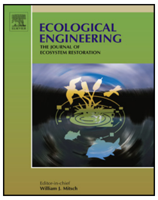

Response of *Nerium oleander* to phosphogypsum amendment and its potential use for phytoremediation

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ABSTRACT

This work was carried out to check the feasibility of a sustainable disposal of phosphogypsum (PG) by application in agriculture. The objectives of this work were to examine the influence of PG amendment on soil physicochemical proprieties along with its potential impact on several biochemical and physiological traits of *Nerium oleander* grown under controlled conditions. *N. oleander* plants were grown on soil substrates amended with PG at rates of 0%, 5%, and 10%. The physicochemical proprieties of soil treated with PG amendment were investigated. The effects of PG on *N. oleander* growth, photosynthesis parameters, nutritional status, osmotic regulator contents, heavy metal accumulation and its potential to use in phytoremediation were also sought. Electrical conductivity, calcium, phosphorus, sodium, and heavy metal contents in soil amended increased in accordance with PG concentration. At 10% PG, soil pH decreased significantly in comparison with the control and soil amended with 5% PG, suggesting that acidity of the substrate influenced the availability of metal ions. Biomass accumulation, photosynthesis, leaf water potential, leaf chlorophyll and carotenoid contents were affected by 10% PG application in comparison with 5% PG and control. PG induced an increase in the rate of hydrogen peroxide production and lipid peroxidation in foliage, indicating oxidative stress. This redox stress affected photosynthesis, leaf water potential, leaf chlorophyll and carotenoid contents, which decreased in response to increased PG doses. However, soluble sugars and proline contents increased in all PG-treated plants compared with controls. *N. oleander* foliage contained increased concentrations of Zn, Fe, Ni and Cr. In contrast, low concentrations of Zn and Fe were found in the roots. The bioconcentration factor values of Zn, Ni and Cr in the root of *N. oleander* L. were greater than 1; which indicates the metals accumulation potential by this species. Translocation factor values of Ni and Cr were less than 1, which shows that Ni and Cr are stabilized in the root part of the plant. According to the metal accumulation patterns of *N. oleander*, this species seems to be valuable for application in the phytostabilization process of soil contaminated with Cr and Ni.

1. Introduction

Phosphate fertilizer is obtained by a wet reaction of natural rock phosphate with concentrated sulphuric acid, producing phosphoric acid as the final product and phosphogypsum (PG) as a major solid waste. 1ton of phosphoric acid is produced with 5 tons of PG waste. Worldwide, PG production was estimated at 170 million tons in 2006 (Enamorado et al., 2009). Tunisia produces 10 million tons of PG yearly (Elloumi et al., 2015a). Currently, only 15% of worldwide PG production is recycled, while 85% is stored in the vicinity of factories in large stockpiles, causing huge environmental problems (Tayibi et al., 2009). PG comprises mainly calcium, small amounts of P and F, certain natural radionuclides and certain heavy metals. Thus, the management of this waste is one of the most serious problems currently faced by the phosphoric acid/phosphate industry. However, there is considerable interest in the application

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of PG to agricultural land. PG has a gypsum content of 85–93% and has a similar ameliorating effect to gypsum on soil acidity and fertility (Li et al., 2015). PG may be used in the improvement of crop yield (Vyshpolsky et al., 2010), increasing available S and P (Delgado et al., 2002) and reducing soil erosion (Zhang et al., 1998). The use of PG as a Ca amendment for sodic soils is of particular interest (Hurtado et al., 2011). Blum et al. (2012) showed that the addition of PG to irrigated plots resulted in an increase in Ca^{2+} and ${SO_4}^{2-}$ concentrations in all soil profiles, whilst Na^+ decreased.

Attention must also be paid to radiological safety and the concentrations of heavy metals in agricultural products when wastes such as PG are used as soil amendments. Restrictions on the agricultural use of PG are due to the presence of heavy metals and natural radionuclides especially ^{226}Ra (Abril et al., 2008; Hurtado et al., 2011). Previous reports have discussed the impact of PG radionuclides on the environment (Rutherford et al., 1994; Abril et al., 2008; Gázquez et al., 2014). Rutherford et al. (1994) reported that one of the main problems of PG piles is the emanation of ^{222}Rn from the alpha-decay of ^{226}Ra. Gázquez et al. (2014) showed that the acidic waters leached from the PG piles to the surrounding environment contain very high concentrations of radionuclides from the uranium series. Abril et al. (2008) reported that the enrichment of ^{226}Ra in the soils at three-decade application of PG as soil amendment produced a significant increment in ^{226}Ra-concentration in soils. Bolivar et al. (2000) concluded that 90% of Po and Ra originally present in phosphate rock remain in PG. The USEnvironmental Protection Agency regulation on PG (64 FR 5574) specifies that agricultural PG must contain an ^{226}Ra concentration of below 370 Bq/kg. Hammas et al. (2013) showed that ^{226}Ra activities found in Tunisian PG remain lower than those found for the majority of PG produced elsewhere: after 10 years of ageing, the ^{226}Ra activity is approximately 212.8 ± 11.6 Bq/kg; when the PG age exceeds 40 years ^{226}Ra activities are approximately 132.19 ± 1.35 Bq/kg.

In addition to the radionuclides, heavy metals in PG may cause some problems in agriculture. Considerable research has focused on the uptake of heavy metals by vegetation (Malik et al., 2010; Zouari et al., 2016). Gupta et al. (2014) reported that the uptake of various metals including toxic elements into the plant is enhanced by several factors (i.e., soil pH and microflora, plant induced pH changes and redox reactions in the rhizosphere, and plant-produced chelating agents and other exudates).

Nerium oleander was chosen for the work described here, in order to examine the transfer of heavy metals from soil to the plant. This plant species is an evergreen shrub, common in the Mediterranean region, and has previously been used to study the distribution of heavy metals in soils from different countries (Sawidis et al., 1995). *N. oleander* has been reported to be a useful bioindicator of Zn and Cu in soils (Mingorance et al., 2007). In addition, *N. oleander* acts as excluder of Al, Ba, Cr, Fe and Pb (Mingorance et al., 2007). This species, therefore, has considerable potential for use in phytoremediation programs (Rufo et al., 2011). This paper presents the results of work conducted to determine the bioavailability of heavy metals in soils amended with PG, and the effect of heavy metals on growth, biochemical and physiological responses of *N. oleander*.

2. Materials and methods

2.1. Collection of samples and preparation

The PG used in this work was obtained from a phosphate fertilizer plant in the city of Gabes, Tunisia. Garden soil was air-dried before being thoroughly mixed with PG to ensure uniform distribution of the amendments. The two defined concentrations of PG (5 and 10%) were applied to the soil before transferring plants to the substrate. Control soils contained no PG.

Dry soils were ground to pass through a 2-mm sieve, and processed for chemical analysis. Soil pH in the different treatments was determined in a 1:5w/v suspension in distilled water using a pH meter (Model EA940, Orion, USA) and conductivity measured using a conductivity meter (Model WTW LF 90). Moisture content was estimated by calculating weight loss after drying at 105 °C in an oven for 24 h. Afterwards, organic matter content was determined on the same samples by loss on ignition after 5 h at 550 °C (Berndt, 2005). Total P was determined by colorimetry using perchloric acid digestion (Olsen and Sommers, 1982). Total nitrogen was determined by the Kjeldahl (1883) method (GerhardtK.B.85 Digestion Unit and kjelflex K.360 Büchi Distillation Unit) and titration (Titronic 97/50). Concentrations of heavy metals were estimated by atomic absorption spectrometry (Thermo-scientific EC 3200), after digestion of triplicate samples in HNO_3–HCl (McGrath and Cunliffe, 1985).

2.2. Plant material and growth conditions

Uniform one month old *N. oleander* plants were transplanted into 3-Lpots containing the various substrate treatments and placed in a temperature controlled greenhouse at 28 °C/18 °C ± 3 °C day/night, with relative humidity of 60–70%. Thirty pots were randomly divided into three groups (control and two PG amendments), each treatment with ten replicate pots. Plants were irrigated with distilled water based on water demand, during the growth period, the irrigation scheduling and water quantity being equal for all treatments. Five pots from each treatment were used for analyses of plant growth parameters. The plants were destructively harvested after 60 days.

2.3. Plant growth measurements

At harvest, plants were divided into foliage, stem and roots. Tissues were washed thoroughly in distilled water, dried on filter paper, and either immediately used for analyses or frozen in liquid nitrogen.

After measurement of lengths and fresh weights, shoots and roots were oven-dried at 65 °C to constant weight and dry biomass measured.

2.4. Determination of metal contents

Metal contents in various plant tissues were analyzed by digestion of dried samples in a 4:1 v/v mixture 62% HNO_3:35% $HClO_4$ (Cica-Merck supra pure). Metal concentrations were determined in extracts using atomic absorption spectrophotometry (Thermo-scientific EC 3200).

The biological concentration factor (BCF) and translocation factor (TF) were calculated in order to characterize the transfer of elements. BCF was calculated as the metal concentration ratio of plant roots to soil (Malik et al., 2010). TF was estimated as the ratio of heavy metals in plant shoots to that in plant roots (Cui et al., 2007; Li et al., 2007).

2.5. Determination of physiological and biochemical effects on plants

Photosynthetic activity (Pn) was measured on leaves selected from the median part of the shoots, with three replicate, well-exposed leaves per plant from two plants, between 10:00 am to 1:00 pm, using a portable gas exchange system (Li-CorInc e 6200, Lincoln, Nebraska USA) (Elloumi et al., 2015b).