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Effects of wastewater quality on Henna (*Lawsonia inermis* L.) germination and seedling growth: a case study, Tunisia

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***Abstract*—** *Water resources scarcity led countries like Tunisia to adopt a policy of water economy by increasing the use of treated wastewater in agricultural irrigation. In this context, we studied the effects of raw and secondary treated urban and industrial wastewaters and fresh waters (distilled and well waters used as control) on the germination and seedling growth of Lawsonia inermis. The seeds were used untreated or pretreated with 0.5% H_2SO_4, concentrated H_2SO_4 and soaked in distilled water prior to germination. Germination was conducted at 25°C during 7 days under conditions of dark and light. Germination rate, moisture content, root and shoot lengths were measured in different experimental conditions crossing seed pre-treatment, water quality and incubation condition. The R software was used to perform three classification factors ANOVA, Pearson correlation analysis and multiple linear regression analysis. Results showed that the best germination performance was obtained when the seeds were pretreated by 0.5% H_2SO_4, watered by treated urban wastewater and exposed to light. Treated urban wastewater had a best contribution to germination rate considered model than fresh water and the other wastewaters types tested. So at the stage of early growth treated urban wastewater could be considered as potent water for Lawsonia inermis irrigation.*

***Keywords*— Germination, raw and treated wastewaters, R software, seedling growth, urban and industrial wastewaters.**

I. INTRODUCTION

Water resources management in many arid and semi-arid regions had a big challenge for a long time due to water shortage problems [1]. To address this problem, many countries such as Tunisia introduced adaptation policies focused on alleviating pressure on conventional water resources, reuse of unconventional water, transfers between basins, desalination and pollution control [2]. Reuse of treated wastewater began in Tunisia in the early sixties, applied in agriculture and progressively being developed in industrial, municipal and urban applications [3]. Currently a total of 112 wastewater treatment plants (WWTPs) are located throughout Tunisia with a wastewater capacity of 300 million m^3 per year and producing a volume of 243 million m^3 of treated wastewater. Only a volume of 60 million m3 were reused, 33% for agricultural irrigation [3].

Physico-chemical and biological wastewater characteristics reflect its environmental impacts and its effects on crops when it is reused for agriculture irrigation. For this, Tunisia has developed a standard for agricultural reuse, the NT 106.03 [4]. Reuse of treated wastewater for irrigation may have many beneficial effects such as the increase of the organic matter content of the soil and the positive effects on growth and yield of different plant species [5, 6]. Taking into account the benefits and drawbacks of the irrigation with treated wastewater leads to consider that some crops are more

inermis L., cereals, forages, fruit trees provided that they are not irrigated by spraying, forest trees, flowers and herbs [13]. The effect of treated wastewater irrigation on plants can be evaluated by standardized biotests used as indicators for wastewater reuse [14]. The crop germination inhibition is one of the approaches used to evaluate the toxicity of an effluent used as potential alternative to fresh water especially for water shortage areas [15]. According to [15] and [16], exposure to urban and industrial effluents had an inhibitory effect on germination by delaying it and leading to reduce the fresh weight of seedlings of several plant species namely *Solanum lycopersicum, Trifolium pratense, Triticum aestivum, Secale cereale , Pisum sativum, Trigonella foenum-graecum, Hordeum vulgare, Brassica juncea, Brassica napus, Coriandrum sativum* and *Nigella sativa*.

Henna (*Lawsonia inermis*) is mainly cultivated in India, the Middle East and the Mediteranean [17]. In Tunisia, this plant has been cultivated since 1400 BCE for many traditional and commercial uses. The total area planted with *L. inermis* in this country was estimated to 500 hectares on 5 million hectares of arable land [18]. The present study aimed to evaluate the application effects of raw and treated urban and industrial wastewater on the germination and early growth of Henna *(L. inermis*) seeds pretreated with 0.5% H_2SO_4, concentrated H_2SO_4 or soaked in distilled water. The experiment was conducted under conditions of light and darkness.

II. MATERIALS AND METHODS

2.1 Sampling: plant material, fresh water and wastewater samples

The *L. inermis* seeds used in this study were collected in 2015 year from a local cultivar of the oasis of Chenini in Gabes southeast Tunisia and stored in hermetically sealed containers under ambient laboratory condition with temperature varying from 15 °C to 35 °C respectively in winter and summer seasons. For the germination tests, fresh water types used as control for comparison were distilled water (pH = 7.07; electrical conductivity = 0.38 mS/cm) and well water (WW) set up in the experimental site of Oued Souhil in Nabeul, Tunisia. The wastewater types used in this study were raw (RUW) and secondary treated urban wastewater (TUW) and raw (RIW) and secondary treated industrial wastewater (TIW). Water and wastewaters samples were collected in polyethylene bottles. Urban wastewater samples were effluents collected from a Tunisian WWTP (activated sludge) located in Hammamet region (Fig. 1) with supply water of 90% domestic and 10% touristic origins. Industrial wastewater samples were collected from a WWTP (facultative pond) of Moknine region (Fig. 1) with 70% of water supply from textile industry and 30% of domestic origin. "Fig. 1" presented the inter-annual mean rainfall of the locations of water sampling points in Tunisia and Table 1 summarized the main characteristics of the studied effluents.

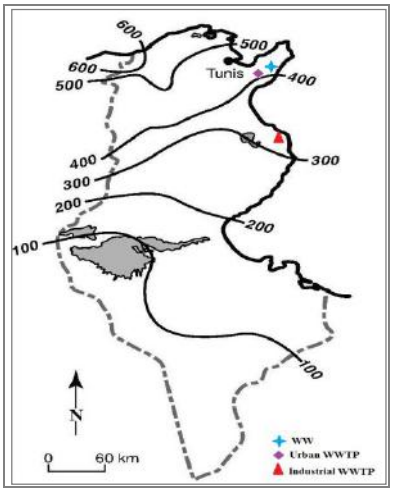

Fig. 1: Map of the inter-annual mean rainfall (mm) of Tunisia with the locations of water sampling points. Notes: WW: well water; WWTP: wastewater treatment plant.

2.2. Physicochemical parameters and methods of analysis

The physicochemical parameters were measured in the Research Laboratory of Valorization of Unconventional Waters in National Research Institute of Rural Engineering, Waters and Forests in Tunis according to Standards Methods. The pH and electrical conductivity (EC) measurements were determined according to the protocols of AFNOR NF T90-008 [19] and NF T90-031 [20] using respectively the pH-meter Adwa 1000 (Romania) and the conductivity-type meter WTW LF330 (Germany). The chemical oxygen demand (COD), biochemical oxygen demand during 5 days (BOD_5) and total suspended solids (TSS) were measured respectively according to the protocols of AFNOR NF T 90-101 [21], NF EN 1899-1 [22] and NF EN 872 [23] in the laboratories of the National Sanitation Utility of Tunisia (ONAS). Chlorides were tittered according to Mohr method [24]. The concentrations of sodium (Na^+) and potassium (K^+) ions were measured by flame spectrophotometry method [25] using the flame photometer Jenway PFP7 (U.K.).

The concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions were determined by complexometric EDTA (Chem-Lab) titration using basic medium [26]. The bicarbonate ions (HCO_3^-) concentration was performed by volumetric titration using sulfuric acid (H_2SO_4)

soaked filter paper in Petri dishes and were irrigated with 2 ml of fresh water and wastewater samples previously described (Fig. 2a). The seeds, irrigated with distilled water and well water, were taken as control. Six replicate were taken for each pre-treatment. Then Petri dishes were placed in growth chamber (Memmert, Germany) at 25°C for 7 days and maintained in the dark. The same experiment was done under light conditions (Fig. 2b). Germinated seeds were counted and germination rate was calculated and expressed in percentage following the formula: G = [NT x 100]/N, where G is the germination rate, NT the number of seedlings emerging on day and N the day after planting [30]. The root and shoot lengths and fresh weight were recorded after 7 days. Dry weight of seedlings was taken after keeping seeds in hot air oven at 80°C for 24 h. The moisture content was obtained by difference between fresh weight and dry weight.

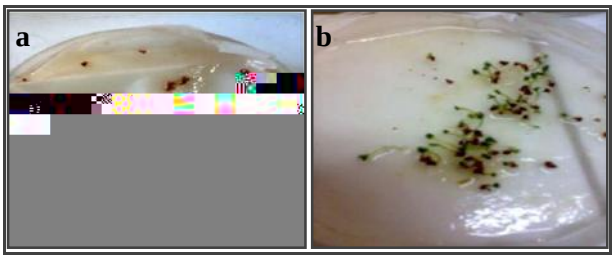

Fig. 2: (a) Lawsonia inermis seeds put in germination; (b) Seeds after 7 days of germination in the light.

2.4. Statistical analysis

The studied statistical model includes three dependant factors (seeds pre-treatment, water quality and incubation condition) explaining the variations of four quantitative variables which are germination rate, moisture content, root and shoot lengths. Data normality of each quantitative variable was assessed through the Shapiro-Wilks test. A three classification factors ANOVA was performed to study the variability of the response variables according to the dependent factors. The relationship between quantitative variables was evaluated E\ 3HDUVRQ¶V FRUUHODWLRQ DQDO\VLV &KDQJHV LQ WKH germination rate were explained and predicted through a multiple linear regression analysis involving the rest of the studied parameters. The multivariate regression analysis model is developed following the equation:

$y = \beta_0+\beta_1 [\ \ « \ \beta_n x_n \ \ 0$ [31] where y the dependent variable, xi the independent variable, β_i the parameter and 0 the error. Statistical analyses were performed using version 3.3.2 of R software [32].

III. RESULTS

3.1. Characterization of fresh water and wastewater samples used for germination tests

The measured physicochemical parameters and their concentrations obtained in fresh water and wastewater samples used for the germination tests are shown in Table 1. All results were compared with standardized levels for treated wastewater quality found in accordance with the Tunisian regulations NT 106.03 governing the agricultural reuse of TWW [4]. Generally, the wastewater collected at the tested WWTPs is slightly alkaline. The pH varies between 7 and 8 with an average value of 7.57. Electrical conductivity (EC) values varied from 2.7 to 6.2 mS/cm with an average of 4.37 mS/cm. Sodium (Na^+) concentrations varied from 547.56 to 1258.58 mg/l with a mean value of 847.42 mg/l. Chloride (Cl^-) levels were between 450 and 1250 mg/l with an average of 1720 mg/l lower than the critical value of 2000 mg/l for the chloride amount in treated wastewater reused in agriculture according to NT 106.03 [4]. Values of the sodium adsorption ratio (SAR) were varying from 18.89 to 36.66 meq/l with a mean of 24.87 meq/l. Potassium (K^+) concentrations varied from 24.5 to 53.11 mg/l with an average of 36.86 mg/l, bicarbonates (HCO_3^-) levels ranged from 504.9 to 1285.2 mg/l with a mean of 1016.28 mg/l and ammonium (NH_4^+) levels were between 3.26 and 40.3 mg/l with an average of 23.74 mg/l (Table 1). Concentrations of the MTE such as Cd^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} were within the 0.001 ±10.7 mg/l interval. Overall, values were below the thresholds accepted of the Tunisian regulations and the only excess was noted for Zn^{2+} in RIW (10.7 mg/l; Table 1). Results showed also that wastewaters used in the biotests presented chemical oxygen demand (COD) levels which ranged from 45 to 1076 mg O_2/l with a mean value of 469 mg O_2/l, biochemical oxygen demand (BOD_5) levels were between 7 and 467 mg O_2/l with an average of 182.5 mg O_2/l and total suspended solids (TSS) levels varied from 7 to 440 mg/l with a mean value of 183.25 mg/l. Only concentrations of COD, BOD_5 and TSS contained in WW and TUW were with the limit of the Tunisian regulations NT 106.03 [4] (Table 1). The comparison of well water and wastewater types tested throughout this study between them showed that well water presented high pH, SAR and chloride values, low COD, BOD_5 and TSS values and high concentrations of Cd^{2+}, Co^{2+}, Cr^{2+} and Pb^{2+} (Table 1). Analyzed urban wastewaters had low pH, EC and SAR values, high concentrations of HCO_3^- and Fe^{3+}, low COD, BOD_5 and TSS values and low concentrations of Cd^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+} and Pb^{2+} (Table 1). The studied industrial wastewaters presented high concentrations of Na^+, Cl^-, Mg^{2+}, Ca^{2+}, K^+ and NH_4^+, very high COD, BOD_5 and TSS values in addition to high concentrations of Cu^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} (Table 1). Investigation of Table 1 indicates that reuse of TUW is acceptable according to pH, EC, COD, BOD_5, TSS, Cl^- and all analyzed MTE concentrations.

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3.2. Water quality effects on germination and seedling growth

The statistical model includes seven variables, three of them are qualitative and four are quantitative. Water quality, incubation condition and seeds pre-treatment explain variations of the independent parameters germination rate, moisture content, root and shoot lengths. "Fig. 3A" showed that the seeds untreated and incubated in darkness did not germinate independently on irrigation water while the maximum germination rate (99.16%) was reached when Henna seeds were pretreated with 0.5% H_2SO_4 under light condition and irrigated with distilled water and treated urban wastewater. The ANOVA test showed that germination rate variation was significant depending on incubation condition ($p < 0.05$) and was not significant on the seeds pre-treatment ($p = 0.07$) and water quality ($p = 0.37$). The interactions between the studied factors water quality, incubation condition and seeds pre-treatment, taken in pairs or thirds, were not significant either ($p > 0.05$).

The moisture content varied from 0 mg when the seeds were untreated and put in germination under darkness condition with all types of irrigation water mentioned above to 0.3 mg when they were pretreated with 0.5% H_2SO_4, put in germination under light condition and irrigated with distilled water (Fig. 3B). Moisture content variations were significant according to the type of seeds pre-treatment (ANOVA, $p < 0.05$) and the incubation condition (at 25°C for 7 days under dark or light) ($p < 0.05$). Whereas there was no significant variations with the irrigation water quality ($p = 0.15$). In addition the interaction of seeds pre-treatment and incubation condition factors had significant effect on moisture content variation of *L. inermis* germinating seeds ($p < 0.05$) but interactions between seeds pre-treatment and water quality; water quality and incubation condition and finally the three studied factors together did not affect significantly moisture content variations ($p > 0.05$).

For root length, the minimum value (0 cm) was obtained when the seeds were untreated and put for germination under darkness condition independently on irrigation water. However pre-treatment of seeds with concentrated H_2SO_4 (18.1 mol/l) for germination under darkness condition and irrigated with distilled water allowed us to reach a maximum length of 0.56 cm (Fig. 3C). The ANOVA test showed that root length did not present significant variations with the three studied factors water quality, incubation condition and seeds pre-treatment taken separately, in pairs or thirds ($p > 0.05$).

Concerning shoot length variations, the minimum value (0 cm) was observed for untreated seeds and put for germination in darkness independently on irrigation water. The maximum value (1.16 cm) was reached for seeds pretreated by distilled water for 7 days, irrigated with distilled water too and germinated under light condition (Fig. 3D). Shoot length varied in a highly significant way with seeds pre-treatment and incubation condition (ANOVA, $p < 0.01$) and significantly depended on water quality ($p < 0.05$). "Fig. 3D" showed that the shoot length of *L. inermis* seeds decreased under all tested wastewater types mentioned above and used for irrigation. Only interaction between seeds pre-treatment and incubation condition factors was significant (ANOVA, $p < 0.05$). Interactions between them and water quality taken in pairs or thirds were found not significant (ANOVA, $p > 0.05$).

Pearson correlation analysis revealed that all quantitative variables (germination rate, moisture content, root and shoot lengths) were significantly correlated to each other. High positive correlations were noted between germination rate and moisture content ($p = 1.2.10^{-12}$, r = 0.81), moisture content and root length ($p = 3.6.10^{-11}$, r = 0.78) and moisture content and shoot length ($p = 2.1.10^{-12}$, r = 0.81). Root and shoot lengths presented very high significant positive correlation between them ($p=2.2.10^{-16}$, r = 0.95).

The examination of the contributions importance of the variables to the model mentioned above revealed that Moisture content showed the best contribution with an absolute value of the coefficient β (beta) of 204.255, followed by the scores received from Root length (β = 49.107), Shoot length (β = 35.198), Incubation condition (β = 27.405), Seeds pre-treatment (β = 2.731) and Water quality (β = 0.302). The regression equation was obtained as follows: Germination rate = ±0.63 ± 0.302 Water quality + 2.731 Seeds pre-treatment + 27.405 Incubation condition + 204.255 Moisture content ± 35.198 Shoot length + 49.107 Root length, where the Germination rate is predicted with certain values of Water quality (DW = 1, WW = 2, RUW = 3, TUW = 4, RIW = 5 and TIW = 6), Seeds pre-treatment (untreated seeds = 1, seeds imbibed in distilled water for 7 days = 2, seeds soaked in 0.5% H_2SO_4 for 48 h = 3 and seeds treated for few seconds with concentrated H_2SO_4 = 4) and Incubation condition (darkness = 1 and light = 2).

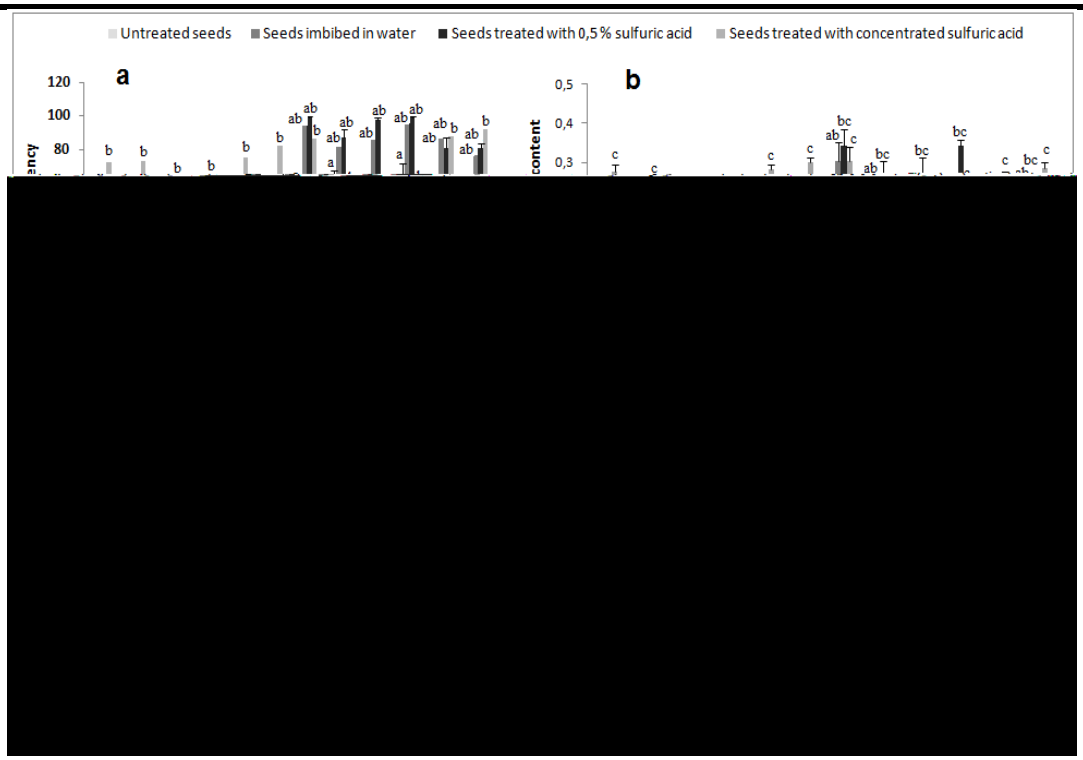

Fig. 3: Effects of water quality, incubation condition (light / darkness) and seeds pretreatment on (a) germination rate, (b) moisture content, (c) root and (d) shoot lengths of Lawsonia inermis seeds.

Notes: DW: seeds irrigated with distilled water, WW: well water, RUW: raw urban wastewater, TUW: secondary treated urban wastewater, RIW: raw industrial wastewater, TIU: secondary treated industrial wastewater. Means (±SD) followed by the same letter are not significantly different at the $p < 0.05$ level, as determined by Tukey's HSD test.

IV. DISCUSSION

Results showed that fresh water and wastewaters used in the germination tests were slightly alkaline with pH values varying from 7 to 8. Park et al. (2014) [33] considered that the shape and availability of nutrients contained in the irrigation water depend on the pH value that it should be between 5.5 and 6.5, values with which the solubility of most micronutrients is optimal. In addition Akinyemi and Souley (2014) [34] reported that irrigation water outside the pH range of 4.5 ±8.5 can cause nutritional imbalance or contain toxic ions. Electrical conductivity (EC) informs about water total soluble salt concentration (Rhoades, 1996). Based on NT 106.03 electrical conductivity threshold, EC values of the analyzed water and wastewaters allows their use for agricultural irrigation. Sodium (Na^+) is one of the ions contributing directly to total water salinity and can be toxic to sensitive crops such as carrots, beans and strawberries [35, 36]. Na^+ amounts of the studied fresh water and wastewaters samples varied from 547.56 to 1258.58 mg/l. The main problem with a large amount of sodium is its effect on the soil permeability and the water infiltration. Irrigation water with a high concentration of Na^+ hardens the soil and makes it compact and waterproof when dry [37]. Based on the literature, sodium, calcium and magnesium cations can be tolerated with relatively large quantities in the water irrigation but a continuous use of water with a sodium adsorption ratio (SAR) expressing the sodium amount compared to calcium and magnesium of more than 9, such as the fresh water and wastewaters samples used in this study (SAR varied from 18.89 to 36.66 meq/l), entails soil destruction [34] resulting in slower water infiltration and reduced soil aeration [38]. When such soils desiccate, crusting is formed, making plowing difficult and opposes the seed germination and seedling emergence [34]. Tunisian standards NT 106.03 for wastewater reuse did not state the potassium limit values in irrigation water but potassium concentrations of the studied fresh water and wastewaters samples included between 24.5 and 53.11 mg/l exceeded the threshold fixed by Peterson (1999) [39] which is limited to 0.2 mg/l. High concentarations of potassium may introduce a magnesium deficiency and iron chlorosis and an imbalance of magnesium and potassium may be toxic, but the effects of both can be reduced by high calcium levels [40]. The chloride anion (Cl^-) concentration of the tested samples varied from 450 to 1250 mg/l and was in accordance with NT 106.03 Tunisian standards [4]. In contrary Ayers and Westcot (1985) [35] fixed a water chloride content limit for

irrigation equal to 300 mg/l and considered that high chloride concentrations in irrigation water increase the osmotic pressure, reducing crop growth due to low water availability to plant. While Karaivazoglou et al. (2005) [41] reported that using irrigation water with high concentration of chloride, an essential element for plant growth, may be beneficial to plant development. There is no limit bicarbonates (HCO_3^-) values used for agricultural irrigation water [4]. According to Peterson (1999) [39] bicarbonates concentration should not exceed 25 mg/l for annual plants. Consequently, the analyzed water and wastewaters (504.9 ± 1285.2 mg/l) did not respect the recommended Peterson limit. Park et al. (2014) [33] reported that the presence of HCO_3^- ions in excess in irrigation water may harm the plant mineral nutrition through its effects on the uptake and metabolism of nutrients, causing unsightly foliar deposits on leaf tissue and precipitates salts. For trace metallic elements, their concentrations in all types of water mentioned above and used for irrigation, did not exceed the values fixed by the Tunisian standard NT 106.03 [4] governing the treated wastewater reuse in agriculture and Peterson (1999) [39] limits. Well water, used as a control, had higher concentrations in Cd^{2+}, Co^{2+}, Cr^{2+} and Pb^{2+} metallic ions and was more saline than the urban wastewater types tested in the present study. This may be due to the fact that the well from which this water was brought is located in the Oued-Souhil region near Nabeul in northeastern Tunisia, constituting since the eighties a management and control of treated wastewater reuse and sewage sludge amendment pilot site [42]. Hussain and Al-Saati (1999) [43] affirmed that irrigation water is the main source of adding salts to the soil and during irrigation with saline water, a leaching of salts directly related to water movement occurs and crops remove only small amounts of salt [44]. Thus in Oued-Souhil region, the groundwater (water wells), will be increasingly saline and will accumulate the MTE contained in treated wastewater used for irrigation. Scientists estimated that parameters such as BOD_5, COD and TSS can provide useful information depending on the final use of reclaimed water [45]. For this, determination of the organic load in the studied wastewaters was performed through calculation of BOD_5 and COD. Gori et al. (2008) [46] conducted experiments to verify the possibility of reusing industrial wastewater of comparable quality to that used in this study (originated from textile (70%) and domestic (30%) activities) for the irrigation of ornamental shrubs. They found that some of them (*Buxus* and *Pistacia*) showed no signs of toxicity, while others (*Viburnum* and *Photinia*) showed greater sensitivity especially during sprinkle irrigation.

It has been proved in this study that germination rate variation of Henna seeds was not significantly depending on irrigation water quality. Thus, raw and secondary treated urban and industrial wastewaters used for irrigation of *L. inermis* seeds did not affect their germination rate. According to Rodosevich et al. (1997) [47] this will not have influence on their productivity. In contrary, seed germination rate, biomass production and root development of other species such as *Pisum sativum*, *Lens esculentum* and *Cicer arietinum* were reduced under irrigation with 50% (an amount of 50% wastewater dissolved in Tap water) and 100% concentrated urban and industrial wastewaters [48]. It is possible that wastewater organic compounds may alleviate some negative impacts. Panasker and Pawar (2011) [49] considered that 20% wastewater concentration does not inhibit seeds germination and seedling growth but at higher concentrations (60%, 80% and 100% concentrations) they are affected. The parameter moisture content did not show significant variations with the irrigation water quality factor. In the literature, seeds moisture content varied with plants species because the plant species groups have different functional traits related to water use like water content, dry matter content and moisture content due to their phylogenetical differences and their different respective life history characteristics [50]. Huma et al. (2012) [16] revealed that the most decrease of seeds moisture content under raw domestic and industrial wastewaters irrigation was obtained for the species *Coriandrum sativum* and *Nigella sativa*. While, the lowest decrease, was observed for *Brassica juncea* and *Trigonella foenum-graecum*. Root length parameter measured at the end of *L. inermis* germination assays did not show significant variations based on the water quality used for irrigation. In contrary Yasmin et al. (2011) [51] showed that the root length was unaffected when *Lens esculentum* varieties was irrigated with raw industrial effluent at a concentration of 10% and an increase of 6.3 ± 11.64% was detected for 20 ± 60% concentration. Huma et al. (2012) [16] stated that this parameter has decreased for the seeds of the species *Brassica juncea*, *Coriandrum sativum*, *Nigella sativa* and *Trigonella foenum-graecum* under raw domestic and industrial wastewaters irrigation. For shoot length parameter, variations were significant depending on the irrigation water quality. Therefore, *L. inermis* shoot length decreased under urban and industrial wastewaters irrigation in both forms raw and secondary treated. These results are similar to those found by Bazai and Achakazai (2006) [52] who studied the effect of treated urban wastewater on germination and seedling growth of *Lactuca sativa* L. and suggested that shoot length decreased with 75 and 100% concentration.

The multiple linear regression analysis confirmed the ANOVA results indicating that germination rates of

Henna seeds did not differ significantly with the irrigation water quality and seeds pre-treatment factors and had significant variations depending on the incubation condition factor. Thus the regression equation presented the factors of irrigation water quality and seeds pre-treatment with lower contributions to the model considered (having respective β absolute values of 0.302 and 2.731) than the incubation condition factor (β = 27.405). Henna seeds presented higher germination rates under incubation in light condition. This confirmed the results approved by the scientists on the light stimulatory effect on seed germination [53, 54]. Small-seeded species like *L. inermis*. need light to avert germination when they are too deep in soil or under plant shadows that could compete with the seedlings and minimize their chance to survive [55]. Hilhorst and Karssen (1988) [56] explained that during seed germination light induces a chain of events leading to gibberellins biosynthesis and enhances the sensitivity of the seeds to these hormones. Concerning the parameters moisture content, root and shoot lengths, they presented also high contributions to the predictive model of Henna germination rate (with respective β absolute values of 204.255, 49.107 and 35.198) and this can be justified by the significant correlation between all quantitative variables (germination rate, moisture content, root and shoot lengths) as reported by the Pearson test results. For seeds pre-treatment factor, it presented a low contribution to the model considered of Henna germination rate. This can be justified by the fact that *L. inermis* seeds were able to germinate without any pre-treatment when they were exposed to light as it was revealed by the results of this study (mean germination rate varied from 44.16% to 64.58% when the seeds were untreated and incubated in light). Indeed Henna seeds have endogenous non-deep physiological dormancy which requires exposure to light in order to break dormancy [28]. Results showed that pre-treatment of Henna seeds facilitate germination even in absence of light and the maximal germination rate was reached when the seeds were pretreated with 0.5% H_2SO_4 (99.16%). Parihar et al. (2016) [28] had pre-treated differently the seeds of *L. inermis* by heating under different temperature regime (20, 25, 30, 35°C for 6 hours in a day), leaching and soaking seeds in water, allowing a germination rate of up to 90%. For the irrigation water quality factor, the results showed that the highest germination rates (20.83% for seeds soaked in DW/incubated in darkness ±99.16% for seeds pre-treated with 0.5% H_2SO_4/incubated in light) were obtained when Henna seeds were irrigated with treated urban wastewater. This can be explained by the fact that they were less saline and less loaded by MTE than well water and raw wastewaters used in the germination tests. Katembe et al. (1998) [57] D Q G % R M R Y L ü et al. (2010) [58] showed negative effect of salinity on seed germination and seedling growth of some crop species such as *Atriplex*, *Brassicaceae* and *Solanaceae* and stated that high NaCl concentrations greater than 400 mM inhibit more seed imbibition, germination and seedling root elongation. Panuccio et al. (2014) [59] demonstrated that saline water used for irrigation of quinoa at low concentrations (100 ±200 mM of NaCl and 13.36 ±53.46 mM of $MgCl_2$) increased the germination rate like pure water used as control.

V. CONCLUSION

The crossing of different experimental conditions namely seeds pre-treatment, seeds incubation condition and water quality (distilled water and well water used as control for comparison and raw and treated urban and industrial wastewaters) used for irrigation, adopted to study the Henna (*Lawsonia inermis*) germination and seedling growth, showed that well water and secondary treated urban wastewater quality was acceptable for use in agricultural irrigation. Germination rate variation of Henna seeds was not significant based on water quality used for irrigation and seeds pre-treatment and was significant according to incubation condition. In fact, the germination performance of Henna seeds was obtained with seeds pre-treatment by 0.5% sulfuric acid, watering by secondary treated urban wastewater and exposure to the light. While, their moisture content variation was significant according to the type of seeds pre-treatment and the incubation condition and was not significant based on the quality of irrigation water samples tested here. Shoot length of *L. inermis* seeds varied significantly with seeds pre-treatment, incubation condition and irrigation water quality. Whereas, root length parameter did not shows significant variations based on these three studied factors. Statistical study showed that irrigation water quality, seeds pre-treatment, incubation condition, moisture content, shoot and root lengths were predictive of the germination rate score. The low contribution of the irrigation water quality factor to the model considered of the germination rate reflected the tolerance of Henna to different types of irrigation water. So *L. inermis* can be considered as a metal-tolerant plant which can maintain its germination under wastewater irrigation condition. At early growth stage, treated urban wastewater could be considered as potent water for *L. inermis*

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