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Habitats, phytochemical metabolites and phytoremediation potential of *Ranunculus sceleratus* L.

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ABSTRACT

Ranunculus sceleratus L. (Ranunculaceae) is an invasive alien widespread species commonly distributed in canals and drains banks. In Egypt, this plant exhibits extensive growth with occasional pure populations in the Nile Delta region. This study aimed to address the drivers for *R. sceleratus* abundance, phytochemical composition, and phytoremediation efficiency. The average abundance of *R. sceleratus* within 60 stands of 25 m2 each was 47.9. The average root and stem lengths were 31.5 and 60.1 cm, respectively. The abundance of *R. sceleratus* was positively correlated with pH, electric conductivity, organic carbon, Fe, Mn, and Zn, while negatively correlated with total dissolved phosphorus, Cu, Co, Cd, and Pb. The shoot had the highest values of all bioactive-metabolites, where phenols, flavonoids, tannins, alkaloids and saponins were recorded 23.16, 5.11, 14.78, 6.34 and 18.50 mg kg-1 dry weight, respectively. *R. sceleratus* had shoot and root bioaccumulation factors (BF) in the following orders: Cd> Ni> Cu> Pb> Zn> Fe> Mn> Co. *R. sceleratus* had BFshoot value of more than one for Ni, Cu, and Pb, while BFroot was greater than one for Cd, Ni, Cu, Pb, Zn, and Fe. Nevertheless, the translocation factor (TF) for all heavy metals were lower than one. Accordingly, *R. sceleratus* is a candidate for phytostabilization and/or phytoextraction tool for the most investigated heavy metals.

الموائل، النواتج الايضية الفيتوكيميائية، وإمكانات المعالجة النباتية لنبات الزغليل

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الكلمات المفتاحية:
المعادن الثقيلة
النباتات المائية الكبيرة
المعالجة النباتية
النواتج الثانوية

الملخص

نبات الزغليل *Ranunculus sceleratus* هو نوع نباتى دخيل غازى ينتشر بشكل شائع على حواف قنوات الرى والصرف. فى مصر يظهر هذا النبات نموا كثيفا ,وتكوين عشائر نباتية منفرده من هذا النبات فى دلتا النيل. هدفت هذه الدراسه الى معرفة أسباب وفرة هذا النبات وكذلك النواتج الايضية وإمكانات المعالجه النباتية للمعادن الثقيلة لهذا النبات. سجل متوسط وفرة هذا النبات 47.9 خلال 60 موقعا، كلا منها بمساحة 25 متر مربع. كان متوسط أطوال الجذر والساق 31.5 و 60.1 سم على التوالى. كانت وفرة هذا النبات مرتبطة بشكل إيجابى مع الأس الهيدروجينى والتوصيل الكهربى والكربون العضوى والحديد والمنجنيز والزنك بينما كانت مرتبطة سلبا مع الفوسفات الكلى الذائب و النحاس والكوبلت والكادميوم والرصاص. كانت أعلى قيم للنواتج الثانوية تم تسجيلها فى المجموع الخضرى للنبات حيث سجلت الفينولات الكليه و الفلافونيدات والتانينات والقلويدات والصابونينات التركيزات التالية 23.16 ، 5.11 ، 14.78 ،6.34 ، 14.78 ، 5.11 و 23.16 ملى جرام/كجم بالوزن الجاف. حقق معامل التراكم الحيوى فى جذر وساق هذا النبات الترتيب التالى: كادميوم> نيكل> رصاص> زنك> الحديد> المنجنيز> الكوبلت. كانت قيمة معامل التراكم الحيوى للمجموع الخضرى أكثر من واحد للنيكل و النحاس و الرصاص بينما كان معامل التراكم الحيوى للجذر أكبر من واحد للكادميوم والنيكل والنحاس

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والرصاص والزنك والحديد. مع ذلك كان عامل الأنتقال لجميع المعادن الثقيلة أقل من واحد. وفقا لذلك يعتبر نبات الزغليل له القدرة على تجميع وترسيب المعادن الثقيلة والتخلص منها.

Introduction

The plant that is undergoing introduction to an environment where it is non-native is called invasive plant [1]. Due to human impacts and increasing soil disturbance, the growth of invasive species exceeds the native plant species [2]. Consequently, the invasive species attained a high abundance that reduces biodiversity and affects the ecosystem [3]. Invasive species are characterized by wide ecological and physiological niches, functional traits that help in modifying the environment [4]. However, globally, invasive species are introduced to provide specific services such as medicinal purposes, fuel, fodder, phytoremediation, etc. [5].

Ranunculus sceleratus L. (Ranunculaceae) is an invasive alien widespread plant species commonly distributed in the canal and drain banks, moist ground, muddy ponds, swamps, wetlands and rivers [6]. In Egypt, *R. sceleratus* exhibits extensive growth with occasional pure populations along the banks of drainage canals in the Nile Delta region. *R. sceleratus* is an annual emergent herb up to 50 cm in height [7]. This plant lives in shallow water and can tolerate infrequent drought. It predominates in the upper layer of hydrosoil/sediment at ca. 15-30 cm depth. The fruiting period begins in April and June while fruiting in late May and August. Numerous previous studies confirmed the medicinal values of *R. sceleratus* as it has a vital role as anti-cancer, anti-diarrhea, antimicrobial, anti-remedy, in addition to its benefit in water purification [6,8].

Nowadays, aquatic hydrophytes are commonly used in phytoremediation techniques such as phytostabilization, phytoextraction, and to monitor water quality [9,10]. Phytostabilization is the ability of a plant to immobilize metal and store it in its underground organs, while phytoextraction is the removal of metal by plant root from soil/sediments or water [11]. Heavy metals are toxic when found in extra-concentrations in the environment and due to their accumulation; they create negative impacts on the ecosystem [12]. As a result of their mobility and solubility, these metals will store within the food-chain. The availability of heavy metals above the acceptable ranges may cause critical trouble in the environment [9]. The role of hydrophytes in monitoring water-quality was usual for decades, but they also able to remove suspended solids, heavy-metals from the contaminated water bodies [13]. In Egypt, few studies were performed on the possible use of *R. sceleratus* in the removal of pollutants. *R. sceleratus* can accumulate numerous heavy metals like iron and zinc from its surrounding environments, and thus, it is applied as an accumulator for these metals [14-16]. *R. sceleratus* is invaluable in the remediation of wetlands and water contaminations [15]. *R. sceleratus* has the potential of phytoremediation and hence accumulates phosphorus, nitrogen, iron and zinc from the sediments and water [6]. Therefore, the chief objectives of this work were to characterize the main factors affecting the abundance of *R. sceleratus* in the polluted drains, estimate its bioactive-secondary metabolites and to assess its phytoremediation efficiency.

Materials and methods

1. Study area and floristic sampling

Sixty-sampled stands (each of 5x5 m) were distributed in the drainage canals of four governorates: Damietta, Kafr-Elsheikh, El-Dakahlia, and El-Gharbia (Fig. 1). These governorates are located within the boundaries of the Nile Delta, Egypt (latitude: 30° 54' N and longitude: 31° 06' E). Climatically, the Nile Delta is positioned in the arid province with a mild winter and a hot rainless summer. In each stand, the list of associated species with *R. sceleratus* was recorded. The identification and nomenclature of the most abundant associated species were carried out according to [17,18]. The abundance was extracted by calculating the importance value index (IVI, out of 200) for *R. sceleratus* and the associated species. The IVI equal the sum of both relative density (number of individuals per unit area) and relative cover (visual estimation) for each species in the sampled stands. The lengths of root and stem of *R. sceleratus* were measured in each stand.

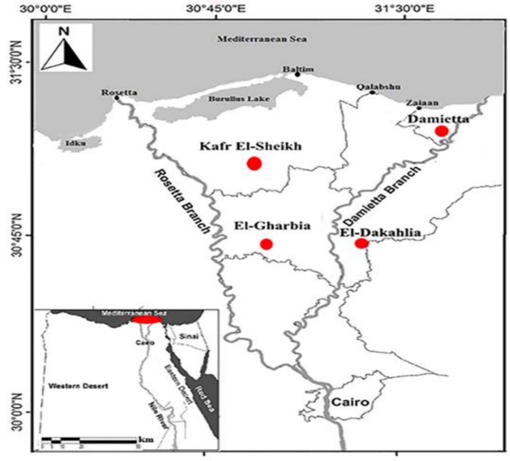

Fig. 1: Map of Egypt shows the location of the Nile Delta region and the four sampling governorates (red circles).

2. Soil analysis

Per each site, a composite soil sample at a profile of 30 cm was collected, then dried and sieved. Soil texture was distinguished using the hydrometer apparatus [19]. pH and electric conductivity (EC) were measured in an aqueous soil-solution (1:5 w/v) using a pH-meter (Apera E190 model), and a conductivity-meter (Apera 209A), respectively. Organic carbon (OC) was estimated in a known weight of dry soil by digestion with chromic acid, sulphuric acid and phosphoric acid, then titration with 1N $FeSO_4.7H_2O$, according to the Walkley-Black protocol [19]. Total nitrogen (TN) was determined by Kjeldahl method while total dissolved phosphorus (TDP) was estimated by direct stannous chloride method [19]. HCO_3 was detected by titration method using 0.1N HCl and methyl orang as an indicator. Cl^- was determined by titration method using 0.01N $AgNO_3$ in presence of potassium chromate as an indicator. Sulphates (SO_4) were gravimetrically determined using $BaCl_2$ solution [19]. In a soil solution (1:5 w/v), Na^+ and K^+ were measured using a flame photometer (PHF 80 B model). The concentrations of Ca^{++}, Mg^{++}, and heavy metals (Fe, Mn, Zn, Ni, Cu, Co, Cd, and Pb) were determined using the atomic absorption spectrophotometer (A Perkin-Elmer, 2380) according to the protocol of USDA/NRCS [19].

3. Phytochemical metabolites and heavy-metals analyses of *R. sceleratus*

The healthy samples of *R. sceleratus* were washed by tap and distilled water to eradicate wastes and debris. Then, *R. sceleratus* plant was separated into shoot and root. The concentration of total phenols in the methanolic extract was estimated using Folin-Ciocalteau reagent [20]. The flavonoids were quantified in aqueous-ethanol extracts using $AlCl_3$ [21]. The concentration of tannins in the methanolic extract was estimated using vanillin-HCl reagent [20]. The level of alkaloids was gravimetrically determined [22]. Saponins were measured using a separatory funnel (aqueous and diethyl-ether) [23]. For heavy metals analysis, one-gram of each dried powder of shoot and root of *R. sceleratus* was digested with concentrated HNO_3 until the digest become clear, then diluted with distilled water until a known volume. By using an atomic absorption spectrometer (240Z AA model), Fe, Mn, Zn, Ni, Cu, Co, Cd, and Pb were measured [24].

4. Phytoremediation potential of *R. sceleratus*

The proficiency of shoot and root of *R. sceleratus* for heavy metals accumulation in soil was calculated using bioaccumulation factor (BF) and translocation factor (TF). BF estimates the capability of plant organs to uptake the metal from the soil (sediment), while TF is the translocation of metal from the roots to the shoots. Here, BF and TF were calculated as follows: $BF_{shoot} = C_{shoot}/C_{soil}$, $BF_{root} = C_{root}/C_{soil}$, and $TF = C_{shoot}/C_{root}$, where C_{shoot}, C_{root}, and C_{soil} indicate the metal concentrations in the shoot, root, and soil, respectively [25, 26].

5. Data analysis

To avoid multicollinearity and over-fitting among soil variables, we applied both Pearson's-correlation ($|r| < 0.8$) and variance-inflation factors (VIFs threshold<5) [27]. Subsequently, 11 variables were retained: pH, EC, OC, TDP, Fe, Mn, Zn, Cu, Co, Cd and Pb. To determine the correlation between the abundance of *R. sceleratus* and the selected soil variables, we applied a scatter response plot depending on the Pearson-linear coefficient (r). All statistical analyses were carried out using the XLSTAT 2018, trial version.

Results

1. Population features and soil characteristics of *R. sceleratus*

The *R. sceleratus* population features include abundance, root, shoot lengths, and the most common associated species (Table 1). The abundance of *R. sceleratus* ranged between 35.6 and 55.2 with an average of 47.9. The root length ranged from 29.3 cm to 37.2 cm with a mean of 31.5 cm. The average shoot length in all sampled stands was 60.1 cm. Moreover, the most common associated species with the highest IVI were *Eichhornia crassipes* (55.4), *Phragmites australis* (43.6), *Typha domingensis* (30.5), *Echinochloa stagnina* (28.8), *Rumex dentatus* (25.4) and *Persicaria salicifolia* (10.5).

Table (1): Population and soil characteristics measurements of *R. sceleratus* in the drainage system of the Nile Delta. Min: minimum and Max: maximum.

Variable	Min	Max	Average± SE
Abundance (IVI)	35.6	55.2	47.9±6.5
Root length (cm)	29.3	37.2	31.5±3.8
Stem length (cm)	51.4	82.4	60.1±4.2
Sand (%)	75.8	85.4	82.7±1.5
Silt (%)	12.2	22.4	15.2±1.1
Clay (%)	1.8	2.4	2.1±0.3
pH	8.1	8.4	8.2±0.3
Electric conductivity (dsm^{-1})	3.5	5.6	4.3±0.7
Organic carbon (mg kg^{-1})	2.3	3.6	1.9±0.8
Total nitrogen. (mg kg^{-1})	3.1	3.9	3.5±0.4
Total phosphorus (mg kg^{-1})	10.7	14.5	12.5±1.8
HCO_3 (%)	0.22	0.26	0.24±0.0
Cl^- (%)	0.16	0.25	0.21±0.0
SO_4 (%)	0.55	0.38	0.32±0.2
Na^+ (mg kg^{-1})	55.6	78.9	66.4±5.6
K^+ (mg kg^{-1})	25.3	34.0	38.8±3.3
Ca^{++} (mg kg^{-1})	12.5	23.5	18.4±2.5
Mg^{++} (mg kg^{-1})	8.2	11.9	10.2±1.6
Fe (mg kg^{-1})	35.2	44.7	40.8±4.8
Mn (mg kg^{-1})	65.2	90.1	85.3±8.5
Zn (mg kg^{-1})	4.6	9.2	5.3±2.0
Ni (mg kg^{-1})	0.9	0.6	0.7±0.1
Cu (mg kg^{-1})	1.2	1.8	1.1±0.5
Co (mg kg^{-1})	0.77	0.89	0.75±0.3
Cd (mg kg^{-1})	0.03	0.06	0.04±0.0
Pb (mg kg^{-1})	1.5	5.8	2.5±1.4

The soil texture of *R. sceleratus* habitat was mainly a coarse-sand fraction (>75%) with little contributions of fine silt and clay fractions (Table 1). The soil pH was slightly alkaline in all of the sampled stands (8.2). The OC was varied from 2.3 to 3.9 with an average of 1.9 mg kg^{-1} dry soil. The average values of TN, TDP, HCO_3, Cl^- and SO_4 were 3.5 mg kg^{-1}, 12.5 mg kg^{-1}, 0.24%, 0.21% and 0.32%, respectively. The average of Na^+, K^+, Ca^{++} and Mg^{++} in soils were 66.4, 38.8, 18.4 and 10.2 mg kg^{-1} dry soil, respectively. Moreover, the trace (heavy) metals in the soil take the following orders: Mn (85.3 mg kg^{-1})> Fe (40.8 mg kg^{-1})> Zn (5.3 mg kg^{-1})> Pb (2.5 mg kg^{-1})> Cu (1.1 mg kg^{-1})> Co (0.75 mg kg^{-1})> Ni (0.7 mg kg^{-1})> Cd (0.04 mg kg^{-1}).

2. The abundance of *R. sceleratus* and soil-factors correlation

The scatter response plot of *R. sceleratus* abundance versus the 11 selected soil-factors indicated positive correlations with some variables (pH, EC, OC, Fe, Mn, and Zn), while the other variables appeared to be negative (TDP, Cu, Co, Cd, and Pb) (Fig. 2).

3. Phytochemical metabolites of *R. sceleratus*

The concentration of bioactive-metabolites of shoot and root of *R. sceleratus* is displayed in Table (2). The shoot had the highest values of all bioactive secondary metabolites, where total phenols, flavonoids, tannins, alkaloids and saponins attained the values of 23.16, 5.11, 14.78, 6.34 and 18.50 mg kg^{-1} dry weight, respectively.

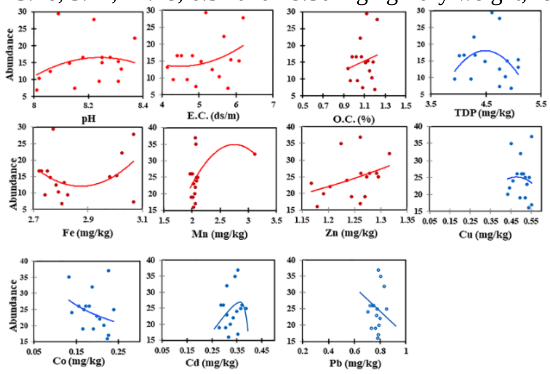

Fig. 2: Scatter response-plot of *R. sceleratus* abundance against the selected soil variables in the drainage canals of the Nile Delta region, Egypt.

Table 2: Phytochemical metabolites (mean value±SE) in shoot and root of *R. sceleratus*

Variable (mg kg^{-1})	Shoot	Root
Total phenols	23.2±1.45	18.5±2.6
Flavonoids	5.1±1.20	4.9±1.12
Tannins	14.8±1.30	10.9±0.90
Alkaloids	6.3±0.85	5.0±1.0
Saponins	18.5±1.20	12.5±1.2

4. Heavy-metals and phytoremediation potential of *R. sceleratus*

The concentration of eight heavy metals in the shoot and root of *R. sceleratus* is illustrated in Fig. 3. The root had the highest concentrations of the studied metals. In both shoot and root, the concentrations of heavy metals consider the following orders: Fe> Mn> Cu> Pb> Zn> Ni> Cd> Co.

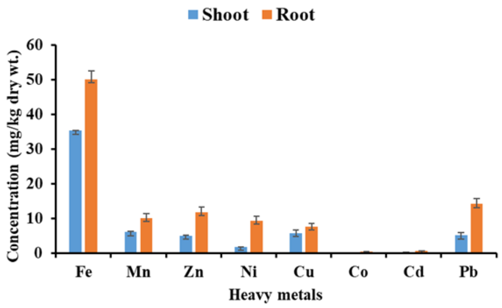

Fig. 3: Concentration of heavy metals (mean value±SE) in shoot and root of *R. sceleratus*.

Both bioaccumulation factor (BF) and translocation factor (TF) were applied to assess the potential of different tissues (shoot and root) of *R. sceleratus* to uptake heavy metals from the soil (Fig. 4). *R. sceleratus* had BF_{shoot} and BF_{root} for the studied metals in the following orders: Cd> Ni> Cu> Pb> Zn> Fe> Mn> Co. *R. sceleratus* had BF_{shoot} of more than one for Ni, Cu and Pb, while BF_{root} of greater than one for Cd, Ni, Cu, Pb, Zn and Fe. Nevertheless, the TF of all heavy metals were lesser than one.

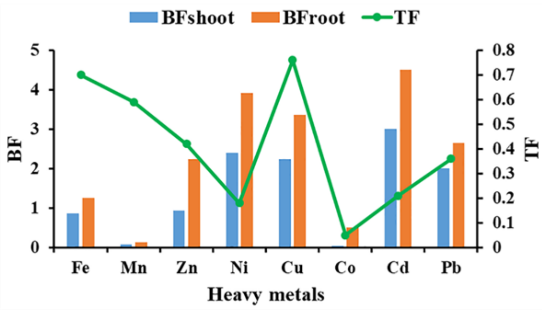

Fig. 4. Bioaccumulation and translocation factors for soil heavy metals in shoot and root of *R. sceleratus*.

Discussion

In this study, the average root length of *R. sceleratus* exceeded the previous study (root length= 10-25 cm) of [6] on the same plant in China. The recorded associates with *R. sceleratus* are completely agreed with [28].

The present study revealed that, *R. sceleratus* preferred coarse soil with a slight alkaline pH. According to the World Health Organization (WHO), the concentrations of Ni, Cd and Pb in soil exceeded the safe limits for the agricultural soils (0.05, 0.003 and 0.1 mg kg^{-1}, respectively) [29].

Several previous studies [28], [30] highlighted the importance of water conditions, dispersal, soil salinity and fertility and interaction with other species on the distribution of plants along with the canal banks habitat in the Nile Delta region. The better habitat features where *R. sceleratus* grows rapidly with a long healthy stem, tall roots and numerous seeds where satisfactory light, soil fertility and water availability [31].

The high concentration of the investigated bioactive metabolites supports the use of *R. sceleratus* in plants-based food or pharmaceutical purposes. Moreover, the increase in secondary metabolites of any plant may be attributed to salinity and heavy metals stress. Secondary metabolites play a vital role in plant life as a tool of adaptation and resistance against the environment and stress [32]. According to Mei et al. [6], *R. sceleratus* is medicinally important against diarrhea, thrombosis, cancer and blood stasis. Moreover, several previous studies reported the ability of Ranunculus spp to synthesize bioactive-metabolites such as phenolics, alkaloids, flavonoids, organic and fatty acids, saponins and essential oils, which collectively contribute to protection against chronic diseases [33-36].

In both shoot and root, the concentrations of heavy metals consider the following orders: Fe> Mn> Cu> Pb> Zn> Ni> Cd> Co. This finding coincided with other previous studies on other macrophytes [37-39], and specifically on *R. sceleratus* in similar closest wetlands habitat [15]. The hyperaccumulation of metals, particularly in roots is a tolerance strategy where plants sequester the high concentration of trace metals in the belowground tissues [38], [40].

Ranunculus sceleratus had BFshoot and BFroot for the studied metals in the following orders: Cd> Ni> Cu> Pb> Zn> Fe> Mn> Co. *R. sceleratus* had BFshoot of more than one for Ni, Cu and Pb, while BFroot of greater than one for Cd, Ni, Cu, Pb, Zn and Fe. Nevertheless, the TF of all heavy metals were lesser than one. This finding was agreed with several previous studies on other aquatic macrophytes [38], [41] and with [15] on *R. sceleratus*. The difference in heavy metals accumulation of the R. sceleratus in this study and any previous studies may be due to other factors such as pollution levels, sampling time, physical-chemical properties of water and finally the method of metals extraction [38], [42]. Similar to previous studies, our study confirmed that, the metal concentration in roots is more than in shoots [40]. The high rate of metals accumulation in roots may follow an exclusion manner and the root has no photosynthetic role and subsequently, this supports plant-tolerance against toxic levels of metals. Moreover, the aquatic macrophytes sequester high contents of heavy metals in their underground tissues through a compartmentalization approach [43]. Farahat and Galal [15] indicated that *R. sceleratus* is an efficient macrophyte able to phytostabilize Mn, Cu, Ni and Pb in the polluted watercourses. Bello et al. [44] stated that, plant species with both BAF and TF greater than one could be candidates for phytoextraction, but species with BAF value>1 and TF value<1 could be appropriate for phytostabilization. Zhou et al [45] reported the potential of *R. sceleratus* to remove Fe, Zn, Cd, Cu, Zn, Pb, Cr, TN, TDP from sewage water. Accordingly, *R. sceleratus* is a candidate for phytostabilization and/or phytoextraction tool for the most investigated heavy metals.

Conclusion

Although *R. sceleratus* is one of the invasive species in Egypt, it is characterized by high concentrations of bioactive-secondary metabolites (total phenolics, flavonoids, alkaloids, tannins and saponins). These metabolites recommend its medicinal importance. On the other hand, *R. sceleratus* is an efficient aquatic macrophyte able to phytostabilize Fe, Mn, Cu, Ni and Pb in the polluted watercourses. In the present study, R. sceleratus is a candidate for phytostabilization and/or phytoextraction tool for the most investigated heavy metals.

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