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RESEARCH ARTICLE

RHOEO DISCOLOR, A MEDICINAL PLANT WITH PHYTOREMEDIATION POTENTIAL.

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Abstract

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Rhoeo discolor is a plant used in traditional medicine mainly due to its anticancer properties. The present work studied, for the first time, its use as a phytoremediation plant. Samples of *R. discolor* were collected in the gardens of the Universidad Juárez Autónoma de Tabasco. *Chrysopogon zizanioides* (vetiver), a phytoremediation plant, was used as positive control. Both plants were exposed for 144 h to leachates from a sanitary landfill for urban waste. Afterwards, they were washed, dried and pulverized. Specimens of each species were left unexposed to leachates to use as negative controls. Elements were identified and quantified by X-rays and by ICP. The elements identified in *R. discolor* exposed to leachates were quartz, $CaCO_3$ and thiocyanate. Arsenic, lithium, Lead, and Thallium were identified at concentrations that were only 1.4 times lower than those found in vetiver, except for Tl (445.71 and 326.33 mg/kg in *R. discolor* and vetiver, respectively). In both evaluated species, exposure to leachates was associated with an increase in the concentration of Cu, K, Mn, Ni and S. In *R. discolor* and vetiver, the concentration of S increased 25 and 6 times, respectively. It was concluded that *R. discolor* has potential as a phytoremediation plant.

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Introduction:-

Rhoeo discolor [syn. *Tradescantia spathacea* Swartz, *Rhoeo spathacea* (Swartz) Stearn] is a plant used in traditional medicine in Mexico. *R. discolor* belongs to the family Commelinaceae and is native to the Caribbean and Central America. In the Mexican southeast, it is known as "purple maguey". Its use as medicinal plant goes back to the 1930s [1], and its anti-cancer activity has been reported since 1963 [2] and as recently as 2016 [3]. Furthermore, this plant contains compounds with antioxidant [4] and antimicrobial [5] activity.

Plants can transform and mineralize a wide variety of organic complexes [6] as part of their natural process and as a function of their autotrophic nature. Plants constitute the biological group with the greatest biosynthetic capacities.

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According to Bragato [7], plants sequester nutrients and store them in their roots and shoots, or accumulate them in other tissues, as in the case of heavy metals, which makes them useful for bioremediation processes.

Phytoremediation is an effective method for the removal of different compounds that can damage or alter the environment. Phytoremediation occupies an important place among the different remediation alternatives because it is an emerging technology based on the combined action of plants (terrestrial or aquatic) and native microbial communities [8]. Phytoremediation takes advantage of the capacity of certain plants to absorb, accumulate, metabolize, volatilize or stabilize contaminants such as heavy metals, radioactive metals, organic compounds and oil products that are present in soil, air, water or sediments. When dealing with organic pollutants, the goal of phytoremediation is the mineralization of substances into non-toxic components (phytodegradation or phytotransformation). Plant enzymes can degrade aromatic rings, polychlorinated biphenyls, phenols and trichlorethylene [8]. The objective of this study was to identify exogenous molecules in the leaves of Rhoeo discolor to demonstrate its potential as a phytoremediation plant.

Materials and Methods:-

Sample collection and processing:-

Specimens of *Rhoeo discolor* were collected in the gardens of the Juárez Autonomous University of Tabasco (UJAT), Villahermosa, Tabasco, Mexico. They were identified in the herbarium of the UJAT. A total of three individuals (*Tradescantia spathacea*) were collected and their leaves separated. *Chrysopogon zizanioides* was used as positive control; it was collected from a sanitary landfill of the company Promotora Ambiental located on the Villahermosa-Teapa road, Tabasco, Mexico.

After an acclimatization period of 168 hours, both plants were exposed for 144 hours to leachates taken from the sanitary landfill, which also receives urban waste. The plants were then washed with distilled water and about 15 g of each individual were air-dried in the shade at room temperature for two weeks. The dry material was pulverized, yielding approximately 5 g of each plant. A control plant (unexposed to leachates) was used for each species.

Pre-treatment:-

Zero point five grams (0.5 g) of each plant sample were placed in jars together with boiling beads for further analysis. Seven ml of HNO_3 and 3 ml of reagent water were added and heated to a temperature of 85 °C for 3 h or until the volume decreased to 4-2 ml. Given the volume of the samples used in this work, 10 ml of a digestive solution of HNO_3 (70%) were added to each sample, a volume equivalent to the volume of acid and reagent water that was added before. The samples were allowed to temper and then adjusted to a volume of 25 ml [9].

The wet digestion method involves decomposition by mixed and unmixed acids in teflon cups placed on aluminum blocks or hot plates [10], or, usually, in precipitate glasses placed on heating plates [11].

Chemical analysis:-

Chemical elements were identified and quantified with an X-ray powder diffractometer (SIEMENS D500). The diffractometer was connected to a computer for data and phase analysis using the Diffract-AT software (version 3.2, Livermore, CA, United States of America, 1995-2000) [12]. An inductively coupled plasma-optical emission spectrophotometer (ICP-OES; IRIS Advantage, Thermo Jarrell Ash Corporation) and the multielement standard solution 4 for ICP (Sigma-Aldrich, St. Louis, United States) were also used.

The data were processed using ANOVA statistical analysis and Fisher's Least Significant Difference (LSD) method with the software STATGRAPHICS Centurion XVI, version 16.1.02.

Results and Discussion:-

X-ray diffraction:-

X-ray diffraction makes it possible to identify the compounds that give natural products certain interesting properties [13].

Figure 1:- shows the results obtained from X-ray diffraction for purple maguey leaves. Three molecules were identified: quartz, calcium carbonate and iron (II) tetrapyridine dithiocyanate.

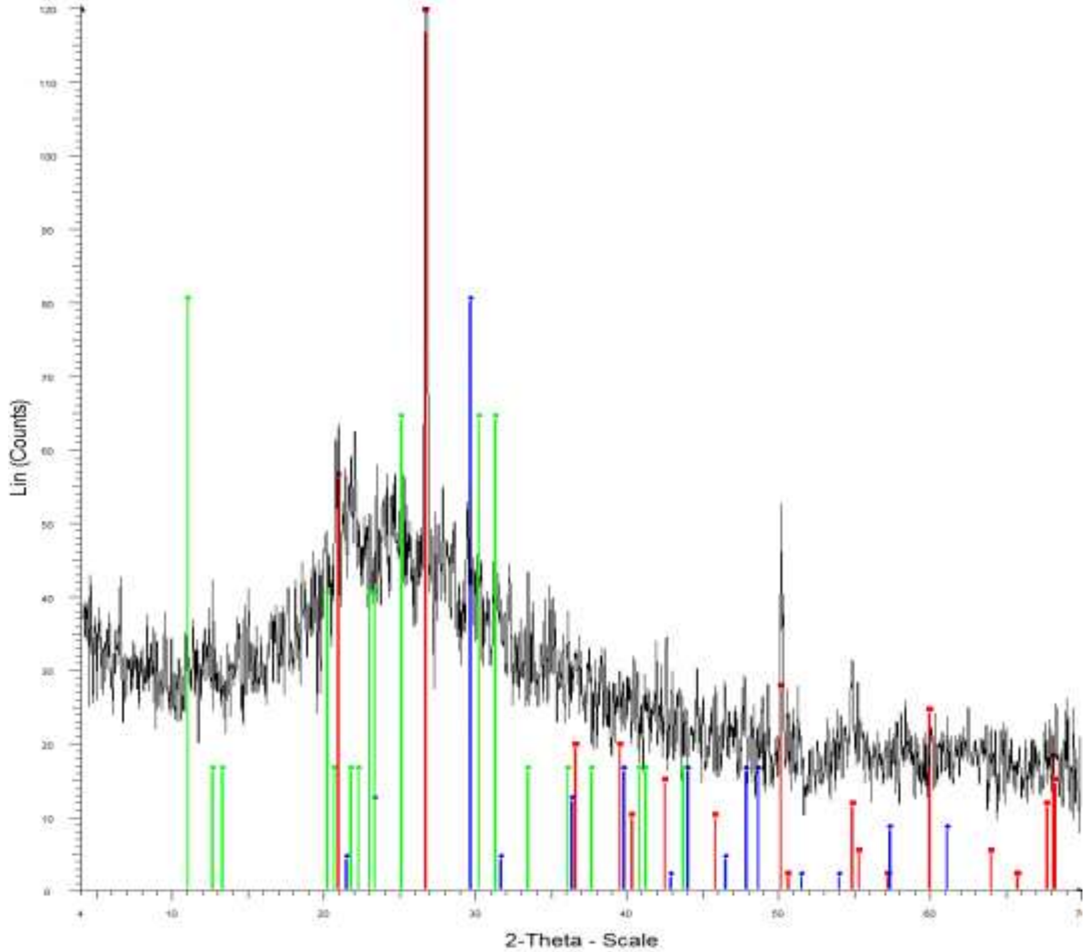

Figure 1. Powder X-ray diffraction pattern at 25 °C. The following compounds: □ Quartz, low alpha SiO_2; □ Calcium carbonate $CaCO_3$; □ Iron tetrapyridine dithiocyanate $C_{20}H_{20}N_4 \cdot Fe(SCN)_2/Fe(SCN)_2 \cdot 4(C_5H_5N)$.

Plants obtain calcium carbonate, an essential compound, from the soil. Calcium is needed for plant growth and for processes of cell division and elongation. This element improves the resistance of plants against pathogens and participates in the development of new leaves, flowers, roots and fruits. A previous study [12] identified ferrocyanide and a derivative of it, sodium nitroprusside, in purple agave. Sodium nitroprusside is a potent vasodilator agent that is used to treat severe cases of arterial hypertension; it is probably responsible for the medicinal properties attributed to purple agave in traditional medicine. Furthermore, the enzyme rhodanese catalyzes the reaction of sodium nitroprusside with thiosulfate to form the metabolite thiocyanate. At the beginning of the 20th century, thiocinate was used in the treatment of hypertension, but its use was abandoned due to the toxicity associated with it [14].

Cyanide is produced by the human body and exhaled with each breath in small concentrations; it is also produced by more than a thousand plant species such as sorghum, bamboo and cassava. Relatively low concentrations of cyanide can be toxic to humans, flora and fauna [15]. A human oral LD50 of 1.1-1-5 mg CN-/kg body weight has been estimated for the ingested NaCN and KCN [16]. In contact with normal skin, the LD50 value is 100 mg/kg of weight [17].

Thiocyanate (also known as sulfocyanate, sulphocyanide or rhodanide), which was identified in purple maguey, is the anion [SCN]− and the conjugate base of thiocyanic acid.This compound was previously known as rhodanide (from the Greek word for rose), because of the red color of its complexes with iron. It is produced by the reaction of elemental sulfur or thiosulfate with cyanide:

$$8\ CN- + S_8 \rightarrow 8\ SCN^-$$
$$CN^- + S_2O_{32}^- \rightarrow SCN^- + SO_{32}^-$$

The last reaction is catalyzed by the enzyme sulfotransferase, also known as rhodanasa or rhodanese, and may play a role in the detoxification of the body from cyanide.

Thiocyanate shares its negative charge almost equally between sulfur and nitrogen. It is an ambiguous ligand and can thus act as a nucleophile in both sulfur and nitrogen. [SCN]- can also act as a bridge between two or even three metals (M-SCN-M; >SCN- or -SCN<)[17].

In the present study, thiocyanate may have come from the leachates or may have originated in the reaction catalyzed by rhodanese in the presence of cyanide [12]. Whichever is true, the results confirmed the initial hypothesis that *R. discolor* has the capacity to absorb and translocate contaminating elements and can thus be considered a phytoremediation plant. This is the first such study on this species; however, further research is required to fully characterize it and improve its economic value. The results of this study can also provide important information to rural populations where this species is consumed frequently, so that people take into consideration the places from which *Rhoeo discolor* plants are obtained for medicinal use. Furthermore, this study could be used to provide advice for the development of backyard plantations with well-characterized species.

Optical spectroscopy:-

Table 1:- Analysis of elements in two species exposed to leachates

Elements	Leaves of contaminated purple maguey	Leaves of uncontaminated purple maguey	Leaves of contaminated vetiver	Uncontaminated vetiver leaves	Total levels (leaves and roots) and thresholds in vetiver
Ace	42.686	ND	59.715	ND	21-72 [18]
Cu	0.546	0.23	0.512	0.895	13-15 [18]
K	4086.939	845.33	1310.91	919.81	35-45 [19]
Li	0.913	ND	1.329	ND	ND
Mn	32.037	11.23	42.094	13.96	4-13 [20] 750 -1110 [19]
Ni	238.686	103.43	204.39	114.41	347 [18]
Pb	0.417	ND	0.443	ND	>78 [18]
S	36.463	1.45	68.523	7.605	ND
Tl	445.713	ND	326.33	ND	ND

Units are shown in mg/kg.
ND. Not available
Truong et al., 2008 [18]. Roongtanakiat, 2009 [19]. Banerjee et al., 2016 [20].

Truong [21] reported Vetiver plants with high tolerance to metals such as As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn. Truong [22] reported As levels of 11.2 mg/kg in the leaves of vetiver. The levels of arsenic detected in this study in the leaves of vetiver were higher in the presence of leachates than those reported by Truong. Vetiver is plant that has been widely studied due to its phytoremediation capacity, in contrast with Rhoeo discolor, which has not been reported to have phytoremediation properties in soils contaminated with metals. A concentration of arsenic of 20.13 mg/kg has been reported in Artemisia abrotanum.

Copper (Cu) was present in low concentrations, at the threshold levels reported by Truong [23], as is shown in Table 1.

Potassium (K) is an alkaline metal that is transported in cationic form. This is the most abundant cation in vacuoles and the main osmolyte in vetiver. It can reach concentrations between 2,000 and 5,000 ppm [24], but the concentration obtained in this study was 4086,939 mg/kg, although higher concentrations have been reported in other studies. For example, Zhang [25] reported that, under controlled conditions, the concentration of potassium in

Pennisetum purpureum K. Schumach x *P. thyphoideum* Rich was 4000 and 20000 mg/kg in roots and leaves, respectively.

Another essential nutrient is nickel (Ni); plants cannot complete their life cycle in its absence and it cannot be replaced by any other nutrient [2,3]. The concentrations observed in other plants are generally between 0.05-5 mg/kg. Symptoms of toxicity appear at concentrations between 25 and 50 mg/kg plant dry weight [26,27]. However, *C. zianioides* accumulated 204.39 mg Ni/kg dry weight from an initial concentration of 41.012 mg/kg (control sample). The concentration observed in the contaminated species approached the threshold level of Ni (347 mg/kg) for vetiver that was reported by Truong [23].

The concentration of Mn in both contaminated species ranged between 32 and 42 mg/kg, within the range reported in other studies (20-300 mg Mn/kg) [28]. Due to the high amount and availability of this metal in the soil, it may have replaced essential elements for plants such as Ca and Magnesium (Mg) [29].

The absorption of lithium by both species, *R. discolor* and *C. zizanioides*, increased when they were exposed to leachates. The concentration of this element was between 0.913 and 1.32 mg/kg, respectively, while under normal conditions (control sample) no lithium was detected.

Lead (Pb) is a highly toxic environmental pollutant; its presence in the environment is mainly explained by anthropogenic activities such as industry, mining and smelting [30]. The concentration of Pb in the contaminated samples was 0.417-0.443 mg/kg, whereas in the control sample no lead was detected. These values did not exceed the threshold of vetiver (>78 mg/kg) mentioned by Truong [21], maybe because the leachates did not have high concentrations of lead or it was not bioavailable to the plants due to its interactions and reactions with other compounds present in the contaminated soil.

Studies of soil treated with EDTA suggest that this compound is necessary for the extraction of Pb due to the low bioavailability of the latter [31].

Another macronutrient is sulfur (S), which, like P, is a non-metallic element. Sulfur is a constituent of the amino acids cystine, cysteine and methionine and, therefore, of the proteins that contain them, as well as of thiamine, biotin and coenzyme A. In the absence of sulfur, proteins cannot be formed [32]. Plants that are grown using hydroponics can usually tolerate relatively high concentrations of S.

The concentration of sulfur in the control samples was significantly lower than in the contaminated samples, ranging between 36-58 mg/kg.

Regarding thallium, the norm NOM-147-SEMARNAT-SSA1-2004 [33] indicates that the reference concentration of Tl in soil under industrial use is 67 mg/kg. This value was exceeded in the samples of soil contaminated by leachates from the sanitary landfill due to the amount of Tl in the leachates (data not shown). The vetiver plant was able to absorb Tl at a concentration of 326.33 mg/kg, while maguey absorbed it at a concentration of 445.713 mg/kg. Although little is known about the accumulation of Tl in *C. zizanioides*, concentrations of 65 mg/kg [34] and 251 mg/kg have been reported in other species [35].

Thallium is a heavy metal that is highly toxic to plants, animals and humans. Zitko [36] reported a LD50 of 0.03 mg/L for Atlantic salmon. In plants, it inhibits the germination of seeds and the formation of chlorophyll. Furthermore, the human body absorbs Tl very efficiently, especially through the skin, the respiratory organs and the digestive tract. Thallium poisoning is usually caused by the accidental intake of rat poison, which contains large amounts of thallium sulphate. Thallium can also be used for ant control, and it has uses in the electronics industry for the manufacturing of semiconductors switches and fuses. This metal is soluble in water and mobile in soil, which explains its bioavailability in soil and leachates.

Chrysopogon zizanioides is a plant species native to India that is used for the phytoremediation of metals [37] due to its capacity to bioaccumulate metals such as cadmium and copper [38]. There are also reports of the translocation of mercury through phytoextraction mechanisms [39]. Abaga et al. [40] reported the effectiveness of vetiver in the absorption and elimination of endosulfan (an insecticide and organochlorine acaricide). Some studies report the use of this plant for the treatment of wastewater [41]. Other important studies on this species have found evidence of the absorption of tetracycline (antibiotic) and mercury using liquid chromatography-tandem-mass spectrometry, with complete elimination of these elements from roots and shoots in forty days [42].

Vetiver has also been reported to accumulate arsenic in soils contaminated with pesticides with different physicochemical properties. The results obtained by Datta et al. [43] show that vetiver is able to tolerate moderate levels of arsenic, up to 225 mg/kg, and can be efficiently used for the removal of arsenic. There are reports of this plant as a "hyperaccumulator" of lead and zinc [44].It has also been used in phytoremediation tests with hydrocarbons [45].

Chrysopogon zizanioides tolerates extreme climatic variations such as prolonged droughts, floods, submersion and extreme temperatures (-15 to 55 ºC). It also tolerates soils high in acidity and alkalinity (pH 3.3 to 9.5), high levels of Al (saturation percentage of 85%), Mn (578 mg/kg), soil salinity (ECSE 47.5 ds/m, and sodicity (ESP 48%) [46,47], as well as a wide range of heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn), especially lead and zinc. *Chrysopogon zizanioides* can also absorb and promote the biodegradation of organic waste (2,4,6-trinitrotoluene, phenol, ethidium bromide, benzo [α] pyrene, atrazine) [48].

This shows that the control species (vetiver) used in the present stud has been widely characterized. In contrast, this is the first time that metals absorbed by *Rhoeo discolor* are identified and quantified. The most important pollutants, in terms of their toxicity, that were observed in purple maguey leaves were arsenic, lead and thallium.

The results obtained here showed that *R. discolor* is a plant with potential for use in the phytoremediation of leachates, in addition to its use in traditional medicine and gardening. This plant may be more efficient than *Crysopongo zizanoides*; however, a possible disadvantage is that it has a lower biomass than vetiver.

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