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# Ecophysiological adaptation and potential of energy production of two halophytes grown in the Red Sea coast of Egypt

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Natural habitats in littoral salt marshes and desert salt marshes along the coastline of the Red Sea are inhabited by several halophytes, which tolerate salinity stress in these areas. Two distinct halophytes inhabiting the Red Sea coast, *Limonium axillare* and *Nitraria retusa*, were studied for their physiological adaptations and energy sources. Results revealed the accumulation of the most abundant soil salts in their tissues. The soils in the study area recorded high concentrations of $Na^+$ and $Cl^-$ compared with other ions such as $Ca^{2+}$, $Mg^{2+,}$ and $K^+$. To combat this stress, the two species absorb salts to adjust the osmotic potential and raise the concentrations of soluble organic compounds – such as proline, soluble sugars, and nitrogen components – in their leaves. In anaerobic digestion, both species yield many organic components that might act as a source to produce biogas. *L. axillare* produced more biogas 277.58 ml $g^{-1}$ TS (361.23 ml $g^{-1}$ VS) compared with *N. retusa* which produced 204.2 ml $g^{-1}$ TS (309.4 ml $g^{-1}$ VS). The presence of soluble organic solutes represents easily breakable molecules in anaerobic digestion. Therefore, *L. axillare* produced more biogas with more soluble carbohydrates (22.15 mg $g^{-1}$ dry wt), proline (13.47 mg $g^{-1}$ dry wt), and soluble protein (33.06 mg $g^{-1}$ dry wt) compared with *N. retusa* which contains less soluble carbohydrates (18.53 ml $g^{-1}$ dry wt), proline (13.64 mg $g^{-1}$ dry wt), and soluble protein (20.04 mg $g^{-1}$ dry wt).

**Keywords:** Anaerobic digestion, Osmotic pressure, Phytoremediation, Salinity, Volatile solids

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## INTRODUCTION

Egyptian coasts stretch over 3000 kilometers along the Mediterranean and Red Seas. The Mediterranean coastline (1200 km) varies from the Red Sea coast due to its geology, geomorphology, and geographic context (Eldeeb & Elemam, 2022). The Red Sea coast of Egypt, including the Gulfs of Suez and ‘Aqaba and the intervening Sinai Peninsula, is about 1,500 km in length. It extends from Suez at the north (Lat. 30° N) to Marsa Halaib (Lat 22° N) at the Sudanian border for about 1080 km with notable variations in the topography, geology, and marine ecology ( Abd El Wahab, 2010; Masria et al. 2014).

The natural habitats in littoral salt marshes and desert salt marshes along the coastline of the Red Sea are inhabited by several halophytes (Salama *et al.* 1999). According to Yasseen and Abu-Al-Basal (2008), halophytes can tolerate salinity stress in these areas through two main strategies: salt tolerance and salt avoidance. Avoidance by elimination mechanisms to lower salt concentrations in the cells or physiological exclusion by root membranes. Some halophytes reduce accumulated ion concentrations in their tissues to maintain saline toxicity in the cell cytoplasm (Dilution mechanism). Such plants can be called salt-tolerant, at which the tolerance mechanisms depend mainly on osmotic adjustment (Adam 1993; Orcutt and Nilsen 2000; Larcher 2003).

The widespread of halophytes with high resistance ability toward salt stress increased the demand for renewable sources of energy production. It is not a new idea to utilize specific plant biomass for the production of biogas (Kinshina *et al.* 2014). In China, the distribution of halophytes in the coastal zone was utilized for producing bioethanol (Liu *et al.* 2012). Rozema and Flowers (2008) revealed that *Salicornia*, *Suaeda*, *Atriplex*, *Distchlis*, and *Batis* species as good sources for biofuel production. Also, Kamel *et al.* (2019) investigated halophytes such *as Avicennia marina, Tamarix nilotica, Zygophyllum album* with *Zygophyllum coccineum* for anaerobic digestion in the production of biogas.

Anaerobic digestion is a widely applied method in treating different residues while providing renewable energy that replaces fossil fuels at the same time. Maize, sunflower, grass, and clover are among the plant species that have been examined for their ability to produce biogas (Dandikas *et al.* 2014), but there is still low interest in halophytic species biomass. Nawaz et al (2023) assessed the co-production of biohydrogen ($H_2$) and biomethane ($CH_4$) by utilizing a less explored halophyte *Atriplex crassifolia*. However, the yield of biogas relies on certain chemical components that can enhance the methanogenesis process such as the amount of salt present in the substrate (De Baere *et al.* 1984). Accumulation of high concentration of salt due to osmotic pressure can cause plasmolysis of microbial cells (Lefebvre and Moletta, 2006; Zhao et al., 2016; Fu et al., 2018), where cation is predominantly responsible for toxicity (Chen *et al.* 2008; Williane et al. 2019). For the substance used for anaerobic digestion it is essential to determine its salt content. Besides, salinity tends to change the chemical constituents in the plant tissues involving the element content and some organic constituents such as crude fiber and water-soluble carbohydrates (Chen *et al.* 2008) reported that some elements such as $Na^+$, $Ca^{٢+}$, $K^+$, and $Mg^{٢+}$ can stimulate or inhibit methane production.

### Description of plants

***Limonium axillare*** (Frossk.) Kuntze belongs to the family Plumbaginaceae. low shrub 20-50 cm; stems erect, leafy, usually branched, the old branches covered with basal parts of dead leaves; leaves oblanceolate, gradually narrowed into a petiole; flowers minute appearing rose-purplish, and white at maturity. *Limonium* flowers from December to May as shown in Figure 1A. It is a widespread species on salt marshes along the coastal regions of the Red Sea, the Gulf of Suez, and the Gulf of Aqaba in Egypt (Boulos 2000).

***Nitraria retusa*** (Forssk.) Asch. (Arabic name: Ghardag or Ghargad) belongs to the family Nitrariaceae. This family comprises of four genera, *Malacocarpus*, *Nitraria*, *Peganum* and *Tetradiclis*, totaling 19 species; but only Nitraria appears in Egypt. *Nitraria retusa* is a native salt-tolerant and drought-resistant shrub with many erect stems, spreading woody branches, fleshy leaves, white-to-yellowish green flowers, and fleshy edible berry-like drupe fruits (Figure 1B). (Täckholm 1974; Boulos 2000). It inhibits three types of habitat: the salt marshes where it forms saline mounds that stud the flat ground of the salt marsh, the less saline sand bars (actual chains of sandy hillocks fringing the shoreline) and the channels of some main wadis near the coast (Kassas and Girgis 1965; Kassas and Zahran 1967).

The present study focuses on the mechanism of adaptation and the ecophysiological responses of two different halophytes (*Limonium axillare* and *Nitraria retusa*) grown on the Red Sea coastline, and to test the capability of the two species as a source for biogas production.

## MATERIAL AND METHODS

### Study area

Two Locations ($L_1$ and $L_2$) along the Red Sea coast were selected as shown in Figure 2. Samples of soil and studied plants were collected from these locations for different chemical and physical analyses. Specimens of plants growing in these areas were also collected, identified, and kept in the Herbarium of Botany Department, Faculty of Science, South Valley University. Six soil samples were collected as a profile at a depth of 0 to 25 cm from each location to analyze soil properties. shoot samples of *Limonium axillare* were collected from $L_1$, while those of *N. retusa* were collected from $L_2$.

### Chemical composition of soils and plants

Collection of soil samples takes place in the area around the root zone of the selected plants, then bagged and transported to the laboratory. The physical and chemical properties of soil, including soil moisture, texture, pH of soil extract, electric conductivity (EC), total dissolved salts (TDS), and mineral content were estimated. Both EC and TDS were determined using a conductivity meter (model 4520 JENWAY UK Bibby Scientific Ltd, Dunmow, Essex); pH using pH-Meter, Jenway, 3305; water content as %). Major cations and anions such as ($K^+$, $Ca^{2+,}$ $Mg^{2+}$, $Na^+$, $Cl^-$, $HCO_3^-$, $NO_3^-$, $PO_4^{3-}$) in soil extracts were estimated as mg/g after Estefan *et al.* (2013), Calcium carbonate ($CaCO_3$) was determined by titration method after Jackson (1967). Organic matter (OM) estimated by ignition method as described by Sparks *et al.* (1996). However, soil texture was determined according to the pipette method (Jackson 1973). Preparation of water plant extract (1:10 w/v) by method after El-Sharkawi and Michel (1977) to estimate ($Na^+$, $K^+$, $Ca^{2+,}$ $Mg^{2+}$, $Cl^-$ and $SO_4^{2-}$ ) by the same methods as described in soil analysis, soluble carbohydrates and soluble protein according to Dubois *et al.* (1956) and Lowry *et al.* (1951). Proline was estimated in dry plant materials according to Ábrahám *et al.* (2010). Determination of moisture content, volatile solids (VS) and total solids (TS) according to Chandra (2009). Total nitrogen (Bremmer & Mulvaney, 1982), crude fibers (AOAC, 2005). Walkley-Black wet combustion method was applied for Organic carbon content (Tan 1996).

### Anaerobic digestion experimental set-up

A batch technique was applied to determine the biogas potential and methane production rate (Hansen *et al.* 2004). Figure 3 displays the experimental apparatus for anaerobic fermentation. The amount of biogas produced in the anaerobic fermentation experiment was measured using the displacing liquid-gathering gas method. The apparatus consists of polyethylene bottles as anaerobic batch reactors with a head cap connected using rubber stoppers with a glass tube and latex tube to another bottle containing alkaline solution for

**Figure 1.** Biomass species investigated, **A**: *Limoniumn axillare,* **B**: *Nitraria retusa*

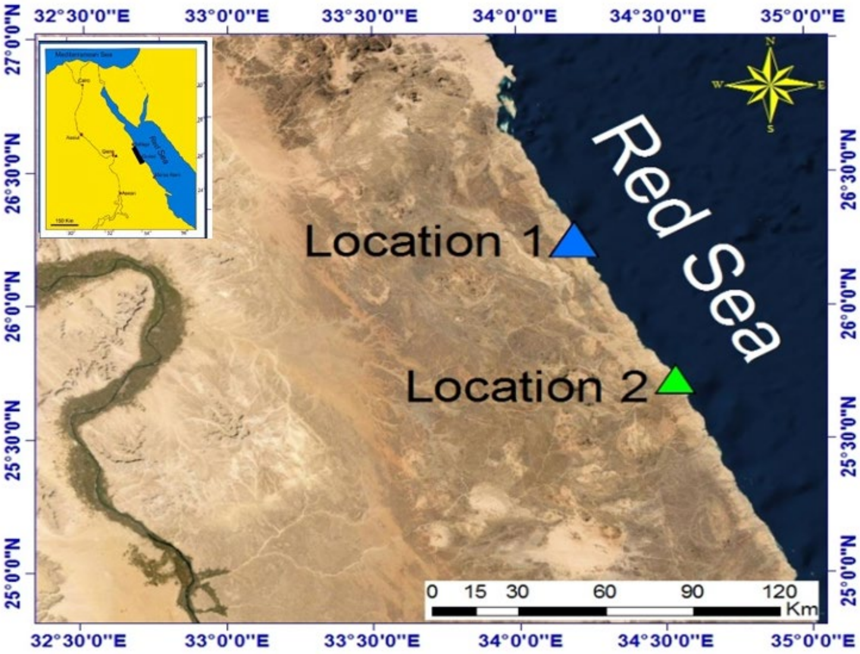

**Figure 2.** Location map of the study area along the Red Sea

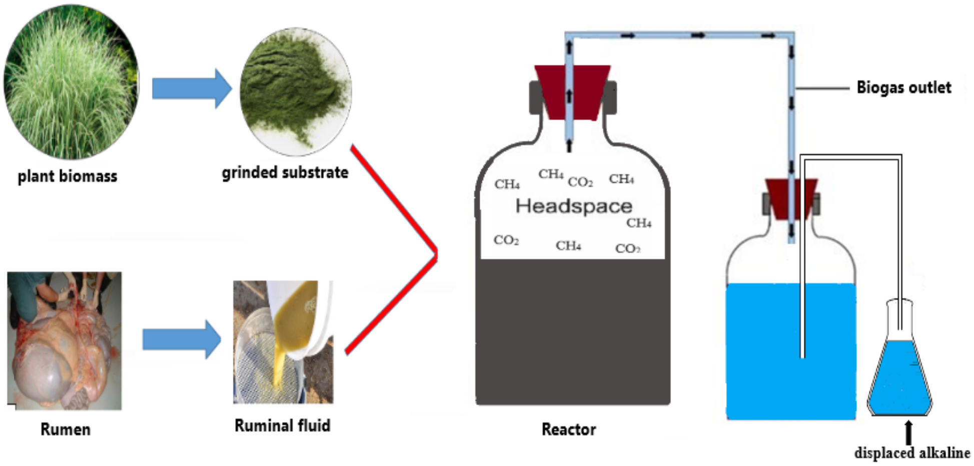

**Figure 3.** Schematic Diagram of Experimental Set-Up

biogas collection. Each alkaline solution bottle is attached to a measuring flask for liquid collection. The batch fermentation experiment was conducted in the water bath at 37±2ºC. Each 1120 ml bottle was loaded with an appropriate amount of digesting substrate with a $VS_{inoculum}/V_{Substrate}$ ratio of 2 to reduce accumulation limitation and to prevent toxicity inhibition (Rico *et al.* 2014) and filled with tap water up to the working volume. Each species was performed in triplicates and three bottles were started for inoculum as blank. The rumen fluid of ruminant animals was utilized as an inoculum because of the high concentration of anaerobic bacteria present in their rumen (Aurora 1983). The process continued for 10 weeks until biogas production became negligible.

Samples of biogas were gathered using a water displacement technique (You *et al.* 2003; Guo 2011). Daily biogas production was measured by displaced alkaline solution in a measuring cylinder. The composition of biogas is made up of a mixture of gases, including methane ($CH_4$) (55-80%), carbon dioxide ($CO_2$) (20–45%), and a small quantity of different gases (Demirbas and Ozturk 2005). By purifying the biogas, incombustible gases like $CO_2$ must be eliminated to increase its energy content and lower compression costs. $CO_2$ absorption in an alkaline solution is used as a purification method by passing the produced biogas through sodium hydroxide (NaOH) 3% (Esposito *et al.* 2012). The biochemical methane potential (BMP) was calculated by subtracting the inherent methane production of the inoculum from the methane volume produced in each reactor, then divided by $VS_{Sub}$ according to the following equation (Elsayed et al., 2015): -

$$BMP_{observed} = \frac{V_{(ino+substrate)} - V_{ino}}{m\,VS_{substrate}}$$

Where BMP observed refers to observed biochemical methane potential (ml $CH_4$/g $VS_{add}$), V (ino + feedstock) is the volume of methane produced by inoculum plus substrate (ml $CH_4$), $V_{ino}$ is the methane volume produced by inoculum alone (ml $CH_4$) and mVS feedstock is mass of volatile solids in the substrate (g $VS_{add}$).

### Data analyses

SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Standard deviations were calculated (SD). Data series were tested for normality with the Kolmogorov–Smirnov test and homogeneity of variance with Levene's test. Differences in chemical composition between species and soil chemical and physical composition were tested using Student *t*-test for independent samples. A significance level of 0.05 was applied for every analysis. The independent t-test, or the two-sample t-test, is a statistical method used to determine whether there is a statistically significant difference between the means of two independent groups

## RESULTS

The regions along the Red Sea coast are exposed to tidal effects with its deposits causing the accumulation of high salts on the coast soil. Table 1 represents the physical and chemical analyses of soil samples collected under *L. axillare* ($L_1$) and *N. retusa* ($L_2$). Soil samples from $L_1$ showed higher concentrations in most of the measured parameters. The remarkable increase of $Na^+$ (72.2 mg $g^{-1}$), $Ca^{2+}$ (10.11 mg $g^{-1}$), and $Cl^-$ (73.83 mg $g^{-1}$) in $L_1$ reflected in higher values of TDS (29.55 g $l^{-1}$) and EC (49.22 mS $cm^{-1}$) compared with $L_2$. Also, $L_1$ is characterized by high organic matter (14.5%) with a high percentage of clay (10.95%) and silt (8.95%). On the other hand, $L_2$ is characterized by increased contents of $CaCO_3$ (18.49%), $HCO_3^-$ (2.44 mg $g^{-1}$) and the soil is 93.2% sand. The lowest values appeared for $K^+$ and $NO_3^-$ in both locations.

By studying the chemical composition of the two plants, results showed an accumulation of both organic and inorganic components in high values due to high levels of soil salinity (Table 2). *L. axillare* showed higher percentages of volatile solids (VS; 80.57%), crude fibers (CF; 18.47%), soluble carbohydrates (22.15 mg $g^{-1}$) and soluble protein (33.06 mg $g^{-1}$) more than *N. retusa*. On the other hand, *N. retusa* accumulated higher contents of ash (33.97%), C/N ratio (56.14), and amino acids. Proline content is almost equal in the two plants (near 13 mg $g^{-1}$). These plants retained the most abundant ions from the soil. *L. axillare* accumulated $Na^+$, $K^+$ and $SO_4^{2-}$ while *N. retusa* accumulated higher concentrations of $Cl^-$, $Ca^{2+}$, $Mg^{2+}$. These results were reflected in recording high TDS value reaching to 18.13 g $l^{-1}$ and so EC (56.14 ms $cm^{-1}$).

The VS content is the main source for biogas production. *L. axillare* gave more VS (80.57%) compared with *N. retusa* (66.03%). Subsequently, *L. axillare* produced more biogas about 277.58 ml $g^{-1}$ TS (361.23 ml $g^{-1}$ VS) compared with *N. retusa* which produced 204.2 ml $g^{-1}$ TS (309.4 ml $g^{-1}$ VS) as shown in Table 3. The cumulative biogas yield obtained from *L.*

**Table 1.** Physical and chemical characteristics of soil samples collected under selected species (average ± SD). t-test for Equality of Means P<0.05

| Soil variables | | $L_1$ | $L_2$ | t-test for Equality of Means | | |
|---|---|---|---|---|---|---|
| | | | | t | df | Sig. (2-tailed) |
| $K^+$ | mg $g^{-1}$ | 0.66±0.11 | 0.48±0.09 | 3.096 | 10 | .011 |
| $Na^+$ | mg $g^{-1}$ | 72.20±22.03 | 41.69±15.21 | 2.792 | 10 | .019 |
| $Ca^{2+}$ | mg $g^{-1}$ | 10.11±2.74 | 3.05±2.97 | 4.286 | 10 | .002 |
| $Mg^{2+}$ | mg $g^{-1}$ | 7.02±1.08 | 5.53±2.22 | 1.481 | 10 | .169 |
| $Cl^-$ | mg $g^{-1}$ | 73.83±20.23 | 40.39±14.71 | 3.275 | 10 | .008 |
| $HCO_3^-$ | mg $g^{-1}$ | 1.47±0.15 | 2.44±1.78 | -1.322 | 10 | .216 |
| $SO_4^{2-}$ | mg $g^{-1}$ | 1.66±0.22 | 1.31±0.24 | 2.521 | 10 | .030 |
| $NO_3^-$ | mg $g^{-1}$ | 0.1±0.01 | 0.16±0.05 | -3.134 | 10 | .011 |
| $PO_4^{3-}$ | mg $g^{-1}$ | 2.67±0.33 | 2.59±0.89 | .193 | 10 | .850 |
| $CaCO_3$ | % | 4.09±0.19 | 18.49±10.13 | -3.480 | 10 | .006 |
| OM | % | 14.50±1.55 | 7.52±2.55 | 5.728 | 10 | .000 |
| pH | | 8.93±0.11 | 8.99±0.15 | -.830 | 10 | .426 |
| TDS | g $l^{-1}$ | 29.55±6.06 | 15.76±4.44 | 4.494 | 10 | .001 |
| EC | mS $cm^{-1}$ | 49.22±10.11 | 26.11±7.30 | 4.539 | 10 | .001 |
| MC | % | 15.50±5.21 | 5.39±3.54 | 3.929 | 10 | .003 |
| gravel | % | 3.44±8.41 | 1.13±0.81 | .667 | 5.092 | .534 |
| sand | % | 76.67±7.58 | 93.20±6.29 | -4.108 | 10 | .002 |
| clay | % | 10.95±3.38 | 1.56±1.70 | 6.083 | 10 | .000 |
| silt | % | 8.95±4.14 | 4.11±4.58 | 1.916 | 10 | .084 |

**Table 2.** Chemical composition of selected species biomass (average ± SD, t-test for Equality of Means P<0.05). MC: moisture content; VS: volatile substances; TDS: total dissolved salts; EC: electric conductivity; C/N: carbon/ nitrogen ratio

| Plant variables | | *L. axillare* | *N. retusa* | t-test for Equality of Means | | |
|---|---|---|---|---|---|---|
| | | | | t | df | Sig. (2-tailed) |
| MC | % | 59.29±1.82 | 70.75±0.88 | -9.836 | 4 | 0.001 |
| VS | % | 80.57±0.25 | 66.03±0.52 | 43.903 | 4 | 0.000 |
| Ash | % | 19.43±0.25 | 33.97±0.52 | -43.903 | 4 | 0.000 |
| TDS | g $l^{-1}$ | 13.50±0.50 | 18.13±1.65 | -4.66 | 4 | 0.001 |
| EC | ms $cm^{-1}$ | 22.53±0.83 | 31.00±1.41 | -8.973 | 4 | 0.000 |
| C/N | | 21.45±0.33 | 56.14±0.95 | -59.811 | 4 | 0.000 |
| Crude Fiber | % | 18.47±0.22 | 4.82±0.43 | 49.309 | 4 | 0.000 |
| Sol. carbohydrates | mg $g^{-1}$ | 22.15±0.69 | 18.53±0.22 | 8.672 | 4 | 0.001 |
| Proline | mg $g^{-1}$ | 13.74±0.71 | 13.64±1.18 | 0.125 | 4 | 0.907 |
| Sol. protein | mg $g^{-1}$ | 33.06±1.58 | 20.04±0.96 | 12.203 | 4 | 0.000 |
| Amino acids | mg $g^{-1}$ | 1.08±0.1 | 3.82±0.1 | -34.293 | 4 | 0.000 |
| $Cl^-$ | mg $g^{-1}$ | 46.28±3.24 | 57.92±2.71 | -4.779 | 4 | 0.009 |
| $Ca^{2+}$ | mg $g^{-1}$ | 4.57±0.81 | 9.12±1.39 | -4.901 | 4 | 0.008 |
| $Mg^{2+}$ | mg $g^{-1}$ | 3.66±0.41 | 8.13±0.59 | -10.792 | 4 | 0.000 |
| $SO_4^{2-}$ | mg $g^{-1}$ | 16.60±0.59 | 12.87±0.82 | 6.426 | 4 | 0.003 |
| $K^+$ | mg $g^{-1}$ | 5.38±0.01 | 2.01±0.01 | 390.075 | 4 | 0.000 |
| $Na^+$ | mg $g^{-1}$ | 107.90±1.23 | 95.64±3.24 | 6.124 | 4 | 0.004 |

**Table 3.** Cumulative methane yields from two selective plants in the anaerobic co-digestion process. (average ± SD, t-test for Equality of Means P<0.05).

| | *L. axillare* | *N. retusa* | t-test for Equality of Means | | |
|---|---|---|---|---|---|
| | | | t | df | Sig. (2-tailed) |
| Cumulative gas production mL $g^{-1}$ VS | 1304.45±161.22 | 1186±120.33 | 4.202 | 4 | 0.002 |
| biogas production rate mL $g^{-1}$ TS | 277.58±57.37 | 204±20.01 | 2.936 | 4 | .043 |
| biogas production rate mL $g^{-1}$ VS | 361.23±50.27 | 309.4±30.04 | 1.566 | 4 | .192 |

*axillare* was 1304.45 ml $CH_4$/g $VS_{added}$ and *N. retusa* 1186 ml $CH_4$/g $VS_{added}$, (Table 3 and Figure 5). Daily methane yield of two wild halophytes from anaerobic co-digestion for 70 days is illustrated in Figure 4. The maximum peak values of daily methane production from anaerobic co-digestion of *L. axillare* with ruminal fluid were 152.5, 231, 145,106, and 132 ml $g^{-1}$ VS on the 2nd, 16th, 18th, 20th, and 24th days, respectively; while the highest peaks in *N. retusa* were 60, 67, 97, 72 and 69 ml $g^{-1}$ VS on 36th, 38th, 42th, 52th and 54th days, respectively. Cumulative biogas yield obtained from *L. axillare* was 361.23 ml $CH_4$/g $VS_{added}$ and *N. retusa* 309.4 ml $CH_4$/g $VS_{added}$, as shown in Table 3.

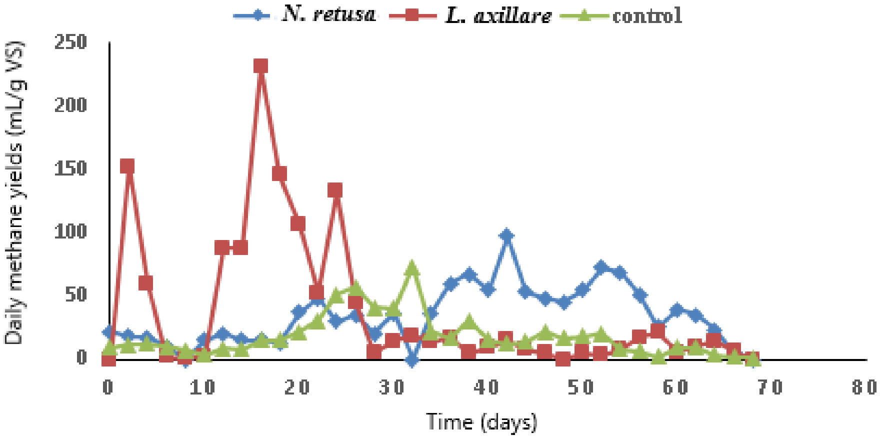

**Figure 4.** Daily methane production during the digestion of *L. axillare* and *N. retusa*.

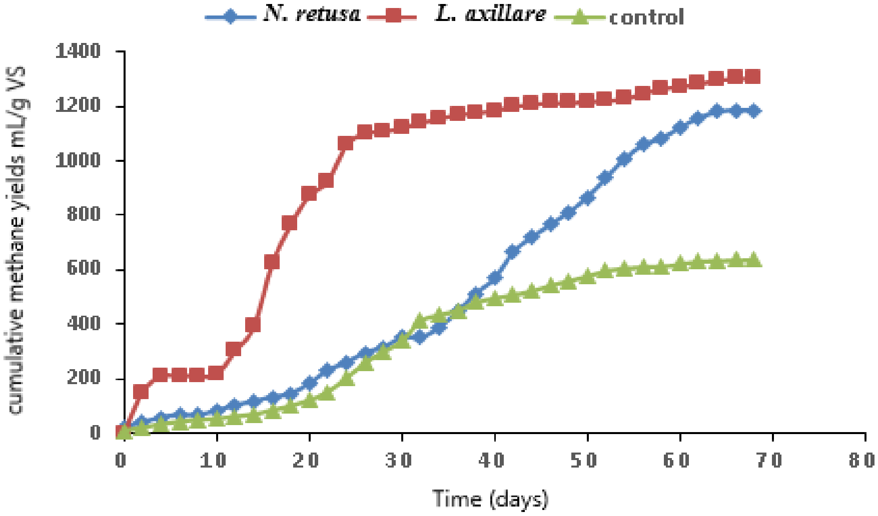

**Figure 5.** Cumulative methane yields produced from the digestion of *L. axillare* and *N. retusa.*

## DISCUSSION

### The physiological adaptation of halophytes against soil salinity

The regions along the Red Sea coast are exposed to tidal effects, with its deposits causing the accumulation of high salts on the earth's crust (Paul and Rashid, 2016). Furthermore, evaporation also plays a role in this salt accumulation; subsequently, the variation in the EC values was detected even within the two locations. Soil analysis showed clearly significant accumulation of salts in the two locations, with variation in organic matter content as shown in Table 1. In location 1, there is a high content of organic compound through clay particles and that is reflected in organic fractions (VS) content in *L. axillare* tissue, compared with *N. retusa* which associated with the low in organic matter in soil in location 2 with high significant difference (t-test, $t = 5.73$ $p<0.05$). However, the high content in salts concentration was reflected in species tissue with a highly significant difference (t-test, $t = 4.49$ $p<0.05$). It is obvious that these plants retained the most abundant ions in their growth medium (Table 2), those ions playing diverse roles in the behavior and physiology of plants in saline environments. Previous researches on halophytes reported that $Na^+$, $Cl^-$ and $K^+$ accumulate in plant tissues to reduce water potential and solute potential to achieve osmotic adjustment (Downton 1982; Flowers and Yeo 1986). The variation in the concentration of $Ca^{2+}$ ions in the two species results from the increase in soil alkalinity due to the accumulation of soluble salts in the soil. Also high concentrations of sodium carbonate reduce the uptake of calcium as previously reported by Day and Ludeke (1993). These results appeared in *L. axillare* tissue which accumulated lower calcium ions than *N. retusa.*

The physiological response of the surviving plants in a certain environment can explain how the plants can adapt to their environmental conditions. Each plant has its own strategy to overcome environmental stress. Plant growth in a saline environment is restricted by three main factors; (i) water deficit, resulting from the low water potential of the rooting medium, (ii) ion toxicity due to excessive uptake of $Cl^-$ and $Na^+$; and (iii) interference with the uptake of essential nutrient especially $K^+$ and $Ca^{2+}$ (Marschner 2012). On the other hand, water stress manifests several biochemical changes leading to an inhibition in the effectiveness of many processes such as the synthesis of proteins, photosynthesis, respiration, and nucleic acids synthesis (Akhtar 2004; Ghai *et al.* 2014)**.** The plant can adapt under salt stress by absorbing ions from the external (as a quick response) and by *a* synthesis of compatible solutes that act as osmolytes, in the long term (Kamel 2002; Hussain *et al.* 2008).

The two halophytes tend to accumulate $K^+$ ions which enhances protein synthesis (Kamel and El-Tayeb 2004; Marschner 2012). Subsequently, protein content was higher in *L. axillare* tissue with a high salinity stress medium compared with *N. retusa.* (Rayan and Farghali 2007) reported that there are two ways that plants react to environmental stresses, i) by increasing their water-binding compounds or ii) blocking the synthesis of proteins from amino acids. Also, they found that some halophytes and xerophytes reach their osmotic adjustment against stress by the role of nitrogen metabolites. Soluble proteins have a crucial role in catching water as bound water under environmental stresses. (Youssef 2009) observed that halophytes tended to retain higher soluble protein, soluble sugar, proline, and total organic osmolytes. Statistical analysis revealed a highly significant variation in soluble protein between the two species (t-test, $t = 12.2$ $p<0.05$) while proline content inside *L. axillare* tissues was slightly higher compared with the accumulated amounts inside *N. retusa* tissues. This proves that the accumulation of proline increases the plants' tolerance to survive a wide range of environmental conditions as pointed out by Aslamsup *et al.* (2011). However, soluble carbohydrates played the main role among other estimated organic solutes in osmotic adjustment. According to Cram (1976), soluble sugars are responsible for up to 50% of the total osmotic potential under saline conditions. That explains the higher soluble carbohydrates content in *L. axillare* compared with *N. retusa.* Statistically, a significant difference in carbohydrates content appeared between the two species (t-test, $t = 8.67$ $p<0.05$).

Both *L. axillare* and *N. retusa* showed higher concentrations of TDS in their cytoplasm because of the accumulation of salts as a quick response, giving a highly significant difference (t-test, $t = 4.66$ $p<0.05$). Droux (2004) pointed out that sulfates have a critical role in biological processes, especially for amino acids and protein synthesis, illustrating their high content in *L. axillare* compared with *N. retusa* with a significant difference (t-test, $t = 6.43$ $p<0.05$).

The studied plants tended to accumulate compatible cations ($K^+$, $Ca^{2+,}$ and $Mg^{2+}$). Accumulation of $Ca^{2+}$ keeps the internal membrane structure exposed to high concentrations of $Na^+$ and $Cl^-$ to adjust osmotic pressure (Nilsen and Orcutt 1996). Calcium also improves potassium uptake under NaCl salinity, as pointed out by Cachorro *et al.* (1994) and Nilsen & Orcutt (1996). $Mg^{2+}$ is an important element in chlorophyll formation, thereby affecting green plant tissues and all bioprocesses (Yasseen and Al-Thani 2007). Chloride and sodium ions are considered the most familiar accumulated inorganic osmolytes. Therefore, the studied species tended to accumulate higher amounts of $Cl^-$ and $Na^+$ to overcome the external salt stress. *L. axillare* is a salt salt-secreting species (Sen *et al.* 2002); it has glands which eliminate salts to the outside of the cells (Salama *et al.* 1999). This character helps liberate from the excess accumulated solutes, especially sodium, as pointed out by Yasseen and Abu-Al-Basal (2008). The ability of *L. axillare* to secrete the salt increased their tendency to depend on the monovalent cations more than the divalent cations. Hence, the accumulated $Ca^{2+}$ and $Mg^{2+}$ in *L. axillare* were lower compared with *N. retusa*.

### The potential of biogas production

Salt stress raised the content of proline, carbohydrates, dietary fiber, energy, protein, and most common components while lowering the moisture content of plant tissue, as the same result recorded by Sarker *et al.* (2018). The chemical composition of the substrate for anaerobic degradation is the most critical criterion controlling the anaerobic digestion process to produce significant amounts of biogas (Kamel *et al.* 2019). Any carbon source can be converted into biogas through anaerobic digestion, yet some organic compounds, like lignin (Tuomela *et al.* 2000; Turcios *et al.* 2016), or inorganic cations that may reduce microbial activity (de Lemos Chernicharo 2007). The VS content is the main source for biogas production. In this manner *L. axillare* had more VS compared with *N. retusa,* subsequently, *L. axillare* produced more biogas compared with *N. retusa*. Although the high significant difference in VS between the two species is not reflected clearly in biogas production and results from the presence of high content in crude fibers in *L. axillare* (18.47%) increasing the presence chance of some indigestible components such as lignin, which can inhibit methanogenesis process.

The presence of soluble organic solutes represents easily breakable molecules for the microorganism in anaerobic digestion (VDI 2006). Therefore, *L. axillare* produced more biogas with more soluble carbohydrates 22.15 mg $g^{-1}$, proline 13.47 mg $g^{-1}$, and soluble protein 33.06 mg $g^{-1}$ compared with *N. retusa*, which contains less soluble carbohydrates 18.53 mg $g^{-1}$, proline 13.64 mg $g^{-1}$, and soluble protein 20.04 mg $g^{-1}$. The low C/N ratio in *L. axillare* (21.45) compared with *N. retusa* (56.14) has a critical role in biogas production*.* Kwietniewska and Tys (2014) stated that Anaerobic degradation depends on the carbon/nitrogen ratio, and the ideal ratio for the anaerobic breakdown of organic waste is between 20 and 35. A decrease in biogas production is predicted due to the high C/N ratio, which indicates that the nitrogen in the medium is rapidly depleting (Jingura and Kamusoko 2017).

The production of biogas increased as electrical conductivity decreased. According to Ogata *et al.* (2016) the salt concentration of 35 mS/cm of EC (dilution, 1:9) reduced methane generation. Salt concentration higher than 80 mS/cm of EC inhibits the production of both $CH_4$ and $CO_2$ and degradation of organic compounds. The high concentration of salts in both species has an inhibiting effect by causing dehydration in bacteria due to osmotic pressure (De Baere *et al.* 1984; Yerkes *et al.* 1997). Increasing concentrations of individual elements such as $Ca^{2+}$, $Mg^{2+,}$ and $Cl^-$ in *N. retusa* had a significant inhibiting influence compared with *L. axillare* These results comply with Chen *et al.* (2008), who reported that individual ions have an inhibiting effect on the methanogenic process. Turcios et al (2021) reported that sodium content of halophytes may have an adverse effect on the anaerobic digestion process, which needs adjustments to achieve stable and efficient conversion of the halophytes into biogas. The toxicity of $Cl^-$, $Na^{+,}$ and $SO_4^{2-}$ in biogas production from halophytes was reported by Akinshina *et al.* (2016). On the other hand, Feijoo *et al.* (1995) reported that the antagonistic effect of cation combinations is greater than that of single cations. Calcium and sodium combinations and calcium, sodium, and ammonia combinations had the best results.

### Daily biogas production rate

Daily methane yield of two wild halophytes from anaerobic co-digestion for 70 days is illustrated in Fig. 4. According to Wang *et al.* (2013), the biogas production rate differ from one plant to another depending on its chemical structure. The biogas production rate was higher and earlier in *L. axillare* than in *N. retusa,* which may result that *L. axillare* is an easier biodegradable substrate than *N. retusa* (Barbanti *et al.* 2014) and has a low inhibiting effect. Cumulative biogas yield obtained from *L. axillare* was 361.23 ml $CH_4$ $g^{-1}$ $VS_{added}$ and *N. retusa* was 309.4 ml $CH_4$ $g^{-1}$ $VS_{added}$, as shown in Table 3 while Fig. 4 represents the daily increase in cumulative biogas yield.

## CONCLUSION

Two halophytes, *Limonium axillare* and *Nitraria retusa*, were studied for their physiological adaptations and energy sources. The two species absorb salts to adjust the osmotic potential and raise the concentrations of soluble organic compounds—such as proline, soluble sugars, and nitrogen components—in their tissues. These compounds help the plants capture water molecules as they attempt to adapt to their surroundings. In anaerobic digestion, both species yield many organic components that might act as a source for the production of biogas. *L. axillare* produced more biogas compared with *N. retusa*. The presence of soluble organic solutes represents easily breakable molecules in anaerobic digestion. Recommendation of this work: wild halophytes would be a promising future for energy production, we may also pay attention to try different wild species in this manner.

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