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Remediation potential of native plant species in Iran to remove diesel from contaminated brownfields

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ABSTRACT

Redevelopment of brownfields sites is a challenging issue throughout the world, which has gained increasing attention in recent years. Lands affected by oil wastes and products are among common types of brownfields sites in Iran. Sorghum (*Sorghum bicolor*) was employed to remediate diesel polluted soils in this study. The main aims of this study were to examine the phytoremediation potential of Sorghum (*Sorghum bicolor*) to remove hydrocarbons from spiked soils as well as the influence of diesel on growth characteristics of the sorghum. Soils were artificially contaminated with 100, 500, 1000, 3000 and 5000 mg/kg diesel. Residual hydrocarbons were measured at different intervals. Establishment of considerable biomass is one critical factor affects phytoremediation potential of plant species which has been addressed in this research. Sorghum demonstrated promising growth behaviour in contaminated soils; however, higher concentrations of hydrocarbons depressed growth parameters of sorghum to some extent. The highest hydrocarbon removal rate was obtained in presence of 100 mg/kg diesel, which was 93.54 % compared to the initial level. Results showed that sorghum is an effective plant that can be used in remediation of hydrocarbon contaminated sites.

Keywords: Brownfields, Diesel, Phytoremediation, Soil, Sorghum.

1. INTRODUCTION

Redevelopment of brownfields sites is a challenging issue throughout the world, which has gained increasing attention in recent years. Brownfields can be defined in different ways in different nations. For instance, the US EPA defines brownfields as a property, the expansion, redevelopment, or reuse of which may be complicated by the

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presence or potential presence of a hazardous substance, pollutant, or contaminant (Lin, 2013; Elrahman, 2016). The number of brownfields is estimated to be more than half a million in the U.S, while in some countries including Iran number and situation of brownfields has not been identified to date. Cleaning up these sites can promote job growth, local tax bases enhancement, takes development pressures off of undeveloped lands and greenfields, protect human health and the environment and improve quality of life (De Sousa, 2003; Li *et al.*, 2016).

The importance of brownfields redevelopment is increasing, particularly in urban areas not only because of the environmental concerns but also due to the scarcity of land in densly-populated areas for various purposes such as residential, commercial, industrial, commercial use and etc. Redevelopment of brownfields sites in many courtiers has frequently reported in recent decade (De Sousa, 2003; Li *et al.,* 2016). However, there are many barriers that complicate redevelopment of brownfields sites in both developed and developing countries such as selection of effective cleaning methods and costs associated with remediation technologies. Other obstacles includes, but not limited to contamination level, liability issues, future land use, capital investments, operation and maintenance issues and lack of enough number of experts in field of brownfields redevelopment in governmental agencies (Elrahman, 2016).

Contaminated sites around oil refineries affected by oil wastes and products in different ways are among common brownfields in Iran in which uncontrolled disposal of oil-related wastes has polluted soil resources over the past decades. Many of these sites which are now inside the cities borders have become abandoned for many years. Redevelopment and remediation of these sites has almost been relinquished mainly due to economical and technical barriers. High cost associated with conventional remediation technologies is a principal obstacle to redevelopment of brownfields in many developing courtiers including Iran.

Using plants and their associated microorganisms which is mainly known as phytoremediation or plan-aid remediation is an emerging green technology that can be a promising solution to restore various brownfiels including hydrocarbon-contaminated sites. Phytoremediation of petroleum hydrocarbon-polluted soils most likely works by a process called rhizosphere degradation or rhizoremediation, in which microbial activity in proximity to plant roots is stimulated by root exudates (Kulakow *et al.,* 2000). Degradation of organic contaminants such as hydrocarbons in the rhizosphere of various plant species has been reported in the literature (Abhilash *et al.,* 2009; Liu *et al.,* 2014; Bisht *et al.,* 2015). The impact of the maize on fuel oil degradation was evaluated in a laboratory study by Chaineau *et al.,* (2000), where the maize seeds were grown in beakers with soil and fuel oil (initial concentration of hydrocarbons was 3300 mg/kg). Their results indicated that in the presence of plants, the removal of hydrocarbons was 20 percent faster than in the non-vegetated soils. Many studies have shown hydrocarbons degradation can occur through phytoremediation successfully (Escalante-Espinosa *et al.,* 2005; Huang *et al.,* 2005; Merkl *et al.,* 2005). However, a few experiments suggest that degradation of hydrocarbons is not considerably increased by the rhizosphere effect (Kulakow *et al.,*

2000). Rhizosphere impact on hydrocarbon breakdown may vary from one plant to another plant.
The most important industry in Iran, i.e. oil industry generates huge amount of oil wastes annually which are mainly dumped or landfilled without enough environmental care. Pipeline fracture and leakage from gas stations are also responsible for intrusion of hydrocarbons to soil. Concentration of hydrocarbons in soil is one critical factor affecting plant establishment in contaminated sites and, therefore, phytoremediation potential of plant species in sites in need of clean up. The main aims of this research were to investigate the influence of different levels of fresh diesel in soil on germination and growth parameters of sorghum (*Sorghum bicolor*) which is a native plant species in Iran as well as its phytoremediation potential.

2. MATERIALS AND METHODS

Bulk sample of uncontaminated soil was collected from lands around the Oil Refinery of Tehran. Air dried soil passed through a 4-mm sieve and mixed thoroughly. Some physico-chemical properties of the used soil are presented in Table 1.

Table 1: Physicochemical properties of the uncontaminated soil

Parameter	Value	Analytical method
Clay (%)	30	Hydrometer measurement
Organic matter (%)	0.9	Walkley-Black
Soil pH	7.4	1:1 soil/water slurry
Electrical Conductivity (dS/m)	3.23	1:2 soil/water slurry
Total N (%)	0.1	Kjeldahl
Phosphorus (mg/kg)	26.2	Olsen

There was no background contamination in the soil. Clean soil was artificially spiked by commercial diesel to gain 100, 500, 1000, 3000, and 5000 mg/kg contamination levels in soil. Samples were landfarmed and mixed using a garden hoe to achieve homogenous distribution of spiked hydrocarbons in soil before placement in pots. Polyvinyl chloride (PVC) pots were filled with 800 grams of the contaminated soil as well as uncontaminated control soil, in triplicate. Sorghum (*Sorghum bicolor*) was cultivated over a 60-day period in greenhouse under sunlight. Monitoring of plant growth was carried out every 20 day; however, germination rate was monitored everyday over the first 15 days of the trial to reach the highest possible rate. Number of seeds germinated in different treatments was counted and expressed as a percentage of the initial number of planted seeds. Shoot biomass and root biomass were also determined every 20 days. Biomass and length of aboveground parts (shoot) and underground parts (root) were also monitored by considering destructive pots. Harvested plants were carefully rinsed with deionized water and oven-dried in 70 °C for 48 hours, then weighed.

In order to measure hydrocarbons concentrations in soil samples, they were initially air dried at room temperature and passed through a 2 mm sieve. Ultrasonic extraction was performed using dichloromethane solvent. Ten millilitres of dichloromethane was added to about 5 grams of contaminated soil and then it was placed in an ultrasonic water bath for three minutes at room temperature. All of these operations were repeated three times (US EPA, 1998). Two μl of the concentrated extract was injected into a gas chromatograph equipped with a flame ionization detector (FID) in order to determine residual hydrocarbon concentration in soil samples. Results were analyzed by one-way using the Statistical Package for Social Sciences (SPSS) 20.0 for Windows, SPSS Inc., IL, USA.

3. RESULTS AND DISCUSSION

Sorghum demonstrated considerable germination and growth rates in presence of different levels of commercial diesel. However, higher levels of hydrocarbons i.e. 3000 and 5000 mg/kg diesel reduced plant growth to some extent. Final germination of plants after 15 days is shown in Fig. 1. Germination rate didn't show any variation after two weeks.

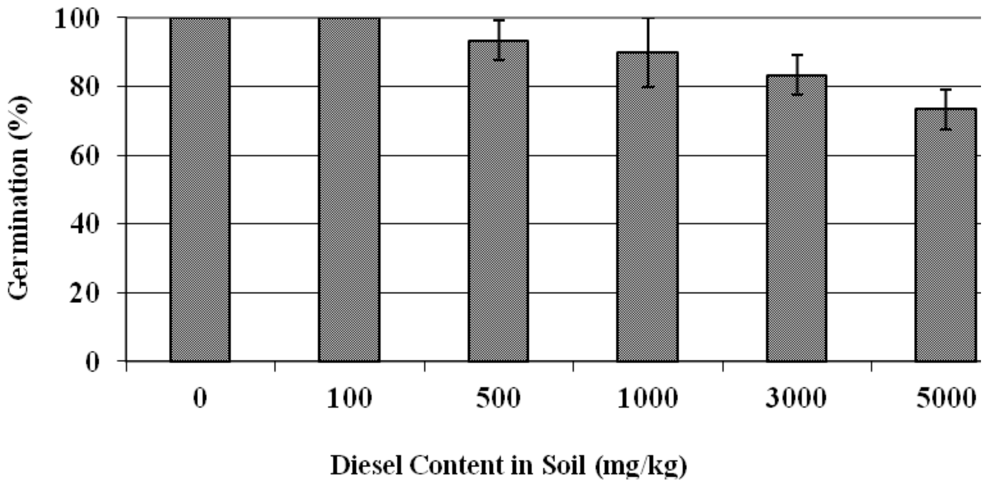

Fig. 1: Final germination of sorghum in diesel-contaminated soils.

Sensitivity of germination as well as initial growth steps of plant species can influence the phytoremediation efficiency (Potashev *et al.,* 2014). Relation between poor germination and subsequent poor plant growth in hydrocarbon contaminated soil has been reported in the literature (Chaineau *et al.,* 1997). Petroleum hydrocarbon pollution did not have significant influence on germination rate of sorghum in soils contaminated with 100, 500, and 1000 mg/kg diesel; however, the subsequent growth was reduced to some extent in presence of 1000 mg/kg diesel in soil. Seedling emergence of sorghum reduced by 26.7 % in the soil spiked with 5000 mg/kg. Seedling emergence, which is more sensitive to freshly added hydrocarbons compared

to aged contamination, can be inhibited or delayed by toxic effects of hydrocarbons. Salanitro *et al.,* (1997) reported seedling emergence reduction of corn, wheat, and oat in soil contaminated with heavy crude oil. Some hydrocarbons can enter into the plant seeds and disturb metabolic reactions or even kill the embryo and inhibit germination, as suggested by Adam and Duncan (2002).
Plant growth parameters in different treatments are presented in Table 2.

Table 2: Growth parameters of sorghum for destructive pots

Parameter	Time (Day)	Initial content of diesel in soil (mg/kg)					
		0	**100**	**500**	**1000**	**3000**	**5000**
Root biomass (cm)	20	3.0	3.0	2.8	2.5	1.9	1.7
	40	6.6	6.4	6.2	5.7	4.7	4.0
	60	10.2	10.0	9.3	8.1	6.8	6.1
Shoot biomass (gr)	20	2.9	2.7	2.6	2.3	1.9	1.6
	40	6.4	6.0	5.7	5.2	4.4	3.8
	60	10.0	9.2	8.6	7.8	7.1	6.1
Root Lenght (cm)	20	18.0	16.0	16.0	13.5	11.8	9.5
	40	33.0	34.0	31.0	26.0	21.4	18.8
	60	46.0	43.0	41.0	38.0	32.0	24.5

Shoot height of the sorghum was also measured over the course of the experiment. Shoot height reduction in all contaminated treatments were less than 50% compared to control treatment, but reduction in shoot height was considerable in presence of 5000 mg/kg diesel in soil, i.e. 41.7 % at the end of the experiment. Reduction in shoot biomass as well as root biomass of sorghum in presence of 100 and 500 mg/kg diesel in soil was almost negligible. In other word, addition of up to 500 mg/kg fresh diesel to soil didn’t affect biomass production of sorghum. Biomass production of sorghum decreased with increasing concentrations of diesel in soil. For instance, addition of 5000 mg/kg diesel to soil reduced dried weights of sorghum root and shoot by 40.2 % and 39 %, respectively, after 60 days, compared to control treatment. Both root biomass and shoot biomass was greatest for the control treatment in which uncontaminated soil was used as expected. Results indicated that sensitivity of shoot biomass is almost comparable with that of root biomass. Both are tolerant to lower levels of diesel in soil, while they are more sensitive to higher diesel content in soil.
After 40 days, root and shoot growth of sorghum showed a decreasing trend. It may be attributed to both inhibition effects of diesel and nutrient depletion in soil, due to lack of nutrient supplement during the experiment. Plant growth rate was also diminished in uncontaminated control soil after 40 days to some extent. Nutrient addition to soil may promote sorghum growth in later stages of phytoremediation experiment. In general, results indicated that sorghum is definitely tolerant to different levels of hydrocarbons in soil up to 1000 mg/kg, while higher levels of hydrocarbons

could reduce sorghum growth parameters considerably. Plant growth reduction in presence of hydrocarbons in soil has been reported in the literature. Chaineau *et al.,* (1997) reported a growth rate reduction of two plant species by more than 80 percent. Gallego-Martinez *et al.,* (2000) also found a reduction of biomass for three plant species supporting the obtained results in this study. Inhibition of growth might be rooted in toxic effects of freshly added hydrocarbons to soil. Diesel usually has more severe effects on plant growth compared to other petroleum hydrocarbon products e.g. heavy crude oil at the comparable levels. Small molecules of hydrocarbons are able to enter and pass through cell membranes leading to reduced membrane integrity or finally death of the cell (Merkl *et al.,* 2004). It may be worth mentioning that initial landfarming and mixing of the spiked soils could lower the effects of low-molecular weight components of diesel in this study compared to researches conducted with real non-spiked soils contaminated with fresh diesel. Shoot biomass establishment is one critical factor controlling phytoremediation potential of a given plant species, but it should be noticed that greater shoot biomass measurements are not necessarily indicator of enhanced remediation (Banks *et al.,* 2003). On-site observations showed that sorghum posses extensive and dense root system. Phytoremediation potential of sorghum in different treatments is shown in Fig. 2. It can be seen in Fig. 2 that hydrocarbon concentrations decreased in all treatments including non-vegetated control treatment. Remarkable impact of sorghum on diesel removal at different sampling intervals was obtained. Natural attenuation could reduce hydrocarbon level in soil contaminated with 100, 500, and 1000 mg/kg by 30.61 %, 30.01 %, and 28.39 %, respectively, at the end of the experiment. Natural attenuation efficiency dropped slightly with increasing concentrations of diesel in soil. Petroleum hydrocarbons were naturally mitigated by only 28.46 % in presence of 5000 mg/kg diesel in unplanted control soil. Natural attenuation of diesel is generally caused by volatilization of low-molecular weight compounds, especially at the beginning of the experiment, as well as soil microbial activity. Sorghum caused a considerably greater hydrocarbon removal compared to unplanted soil in which hydrocarbons were attenuated naturally.

Plant-aid remediation of organic pollutants such as hydrocarbons benefit from the synergy between soil bacteria and plant roots that leads to increase in rate of degradation of persistent and toxic compounds. Positive effects have thus been cited for a wide range of molecules ranging from BTEX (benzene, toluene, ethylbenzene, and xylenes) and simple aliphatics in light crude oil and fuel (Abhilash *et al.,* 2009) to more complex and highly persistent contaminants such as high molecular weight polycyclic aromatic hydrocarbons (Johnson *et al.,* 2005; Liu *et al.,* 2014; Xiao *et al.,* 2015), pesticides, and explosives (Van Aken, 2009). The highest removal in presence of different levels of hydrocarbons in soil was obtained at the end of the experiment for all treatments. Sorghum finally reduced diesel content of soil by 93.54%, 92.2 %, and 91.18 %, respectively, in soils contaminated with 100, 500, and 1000 mg/kg compared to the initial concentrations.

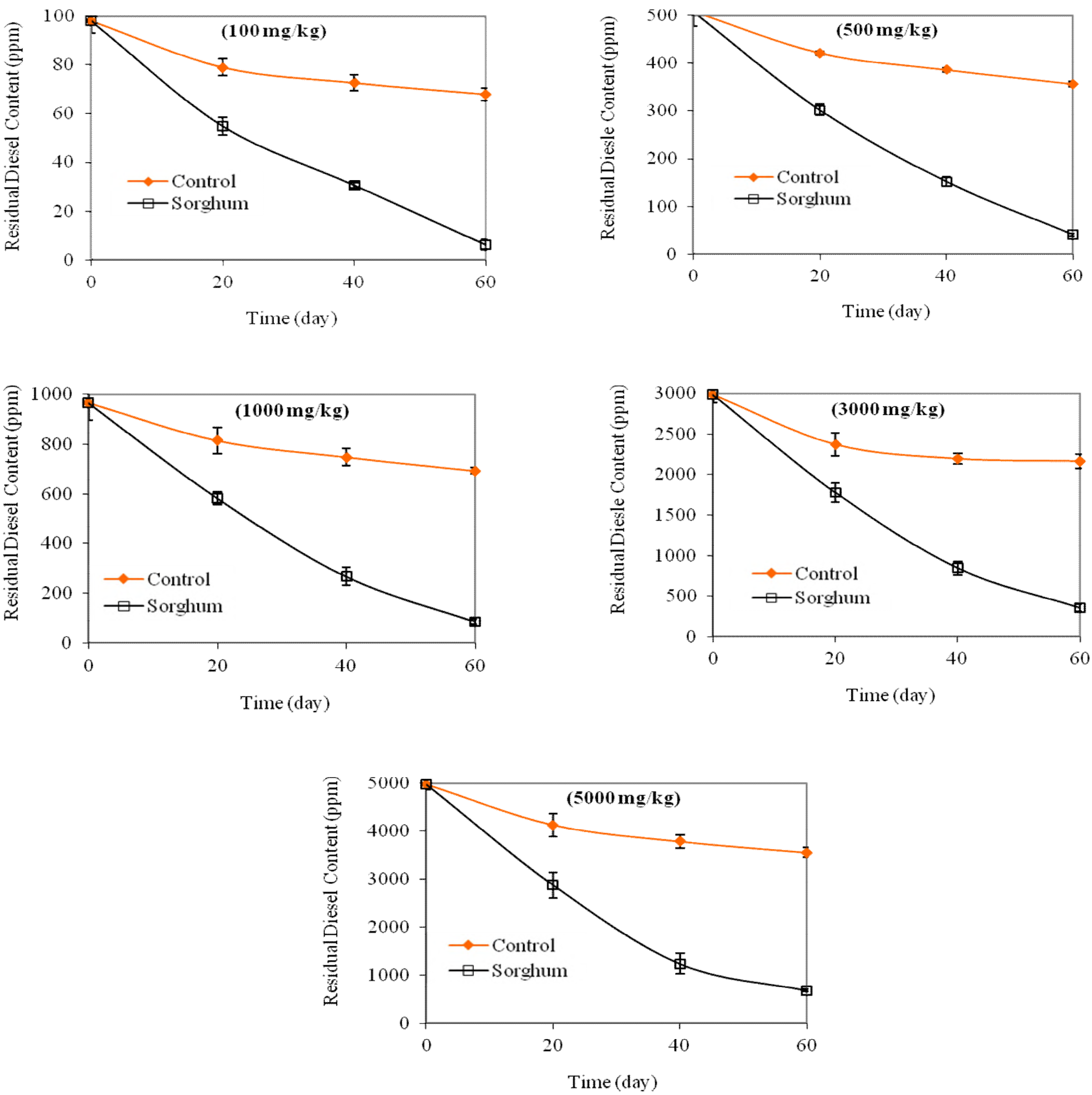

Fig. 2: Residual amounts of hydrocarbons in soil during the experiment under various scenarios.

Phytoremediation effectiveness decreased slightly at higher concentrations of diesel i.e. 3000 and 5000 mg/kg. Phytoremediation potential of sorghum in presence of 3000 and 5000 mg/kg hydrocarbons in soil decreased to 88.18 % and 86.35 %, respectively. In other words, for all contents of hydrocarbons the phytoremediation potential was not dropped below 86%. Reduction in phytoremediation potential at higher concentrations may be attributed to both the toxic effects of hydrocarbons in higher contents and their influence on plant and associated microbial biomass production. Stimulation of soil microorganisms is the principal mechanism by which hydrocarbons are degraded and removed. The remediation process in which hydrocarbons are transformed by microorganisms in the rhizosphere zone (i.e., the microbe-rich zone in close contact with the root system of a given plant) is referred to rhizodegradation or phytostimulation (Cebron *et al.*, 2010; Bisht *et al.*, 2015). Thus it can be assumed that higher root biomass, as obtained for lower hydrocarbon contents, means a larger rhizosphere for microbial population and it is correlated with a higher degradation of hydrocarbons in soil (Merkl *et al.,* 2005).

Fig. 2 reveals decrease in hydrocarbon removal rate after 40 days in all panted treatments that may be caused by plant growth reduction. Another possible reason is a probable change in composition and amount of root exudates at later stages of sorghum growth (Chen *et al.,* 2016). Merkl *et al.,* (2005) suggested that degrading microorganisms are stimulated in their growth and activity by root exudates that vary with plant age and nutritional status. Quantitative and qualitative change in of plant exudates with plant age can affect population and activity of hydrocarbon degrading microorganisms in the rhizosphere, therefore, affect phytoremediation efficiency of a given plant species. Non-appreciable TPHs removal was also observed in a phytoremediation study by Escalante-Espinosa *et al.,* (2005) from 120 to 180 days of culture in planted and unplanted treatments. Hutchinson *et al.,* (2001) and Escalante-Espinosa *et al.,* (2005) observed the highest phytoremediation rate at the first stage of culture in their experiment (up to 60 days), which is almost comparable with the obtained results in the current research.

4. CONCLUSIONS

Results demonstrated that sorghum is a promising plant for phytoremediation of diesel contaminated soils, especially for low to moderate contamination levels. Relatively high reduction of hydrocarbons in presence of sorghum may be attributed to its suitable tolerance and growth in contaminated soil used in the current research. However, sorghum growth and phytoremediation potential were reduced to some extent in presence of 3000 and 5000 mg/kg diesel in soil. Sorghum showed best shoot and root biomass production in contaminated soil with 100 mg/kg that also caused highest hydrocarbon dissipation compared to unplanted soil suggests that greater root and shoot biomass is likely to be associated with higher microbial population and activity in the rhizosphere zone and, hence higher remediation performance. Sorghum is well-known and easy to access plant species in Iran and many other countries. Obtained results indicated that soil remediation with these plant species can be a promising approach to manage diesel contaminated brownfields. It is suggested to investigate the influence of higher contents of diesel in soil on sorghum growth parameters and its clean up performance in future studies. Conducting field trials can also yield more insight into the real-world performance of sorghum under various scenarios.

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