

The assessment of efficiency of saponin as bio-surfactant in removal of nickel and vanadium from soil contaminated by petroleum, Case study: Ahwaz oil pumping unit

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Abstract: This study aims to evaluate efficiency of saponin, a bio-surfactant, in removal of heavy metals like nickel and vanadium from the soils which are smeared by crude oil and it was conducted based on the soil properties of Shahid Chamran oil Pump Station in 2016. For this purpose, after conducting primary studies and in order to determine pilot conditions of test, soil properties were initially studied in this zone and then 5 sampling stations were selected. Parameters of nickel and vanadium were measured along with the TPHs and pH. The studied phases included three variables of temperatures within ranges of (15, 25, 35°C), pH range (6, 7, 8) and concentration of oil compounds within ranges of 10000 and 30000ppm. The findings from assessment on efficiency of removal of heavy metals showed that the conditions with variables of temperature (35°C) and pH (about 9) included the best efficiency of removal of nickel and vanadium. Under concentration 10000ppm, the efficiency for removal of nickel shows rate of 42.98% at constrained mode for removal of nickel and 32.46% to efficiency for removal vanadium. In concentration 30000ppm, rate of efficiency for removal of nickel was 44.34% and also yield of 35.24% for removal of vanadium. This indicates by rising of concentration in oil compounds, the rate of efficiency increased in efficiency of soil washing.

Keywords: Soil contamination, Oil compounds, Soil-washing, Saponin, Fuzzy logic.

1. Introduction: Nowadays, advanced community still relies on using petroleum hydrocarbons to produce needed energy. Despite recent technological advancements, soil contamination occurs frequently due to effect of random crude oil leakage and the given refined products during usual extraction, transportation, storage, refinement and distribution operations (Zhou et al. 2001). Therefore environmental population by petroleum and the related derivatives are assumed as a serious problem throughout the world. Overall, accumulation of pollutants in soil may exert destructive effects on environment and health of human. The existing pollutants in soil may enter into nutrition cycle and seriously jeopardize health

in animals and humans (Merkel et al. 2004). On the other hand, following to penetration of oil materials into the soil there is also a possibility for contamination of underground waters. After entry of petroleum hydrocarbons into soil they compete with climate to be replaced into the bores (Leo Chyojin 2010; Matheney cole, 1994).

With huge amounts of oil and gas reserves, Khuzestan province is deemed as one of the foremost oil centers in Iran. Drilling of oil and gas wells, subsequent oil and gas extraction in this province, pumping of them into refineries and transportation of oil products to places of consumption have caused contamination of ecosystems, especially soils. At present, it is necessarily felt a requirement for prevention

from spreading of these contaminations and adjustment of soils smeared by oil materials. Presence of contaminated soils is deemed as a serious risk for environment and correction of them is an inevitable task (Sharifi Hosseini et al. 2009). Improvement in biological revival of smeared soils by gasoline in 2002 for 90 days was investigated by bio-surfactant. The results of this study showed that about 80% of hydrocarbons were removed in parallel during first 60 days. The maximum removal belonged to reactor treatment (Rahman et al. 2002).

In a survey, sandy soil was washed with initial contamination rate (1000ppm) up to 20 times greater than blank volume by anionic surfactant (JBR425) and cleaning efficiency was reported 67% by this rate of entry of surfactant (Mulligan et al. 2003).

The optimal conditions were examined for the smeared soil by petroleum with bio-surfactant solutions in several extensive studies. The results of experiments indicated that in all modes, optimal conditions have been realized at temperature 50°C and washing period (10min). Similarly, SDS synthetic surfactant and Rhamnolipid (microbial surfactant) and saponin (bio-surfactant) included removal efficiency at approximate level of 79% under such conditions (Urum et al. 2003). In another investigation, the role of additives was explored by means of oil removal and two factors i.e. causative soda and polymers were assessed in removal of oil hydrocarbons (Nedjhioui et al. 2005).

In a study that was carried out under title of analysis on effect of temperature and pH of distilled water on percentage of removal of mercury and chromium as pollutants from contaminated soil by soil-washing, it was shown that the maximum efficiency for removal of mercury and chromium at temperature 45°C was 31.54% and 48.72% respectively (Gholampour et al. 2014).

In a survey titled removal of petroleum from soil by the aid of bio-surfactant, effect of Saponin as a bio-surfactant was examined on removal of petroleum from soil with pollution concentrations 30000ppm and 10000ppm in a soil column at height 15cm and 9cm diameter after 72h. The results indicated that the optimal modes of removal took place at pH=11 in both concentrations and optimal concentration of surfactant solution was 0.1 Wt% for pollution concentration 10000ppm and 0.2Wt% at concentration of 30000ppm also with efficiency

of removal of petroleum at levels 69% and 72% respectively (Seyed Razavi et al. 2011).

Various techniques have been proposed for removal of oil contaminants in soil and they are classified into three physical, chemical, and biological groups. Use of surfactant is one of the chemical methods for removal of oil contaminants from soil. Soil-washing by surfactants is one of chemical techniques which are employed for refinement of contaminated soils by heavy metals, multi-cyclic aromatic hydrocarbons, pesticides, semi-volatile materials and Polychlorinated Biphenyls (PCBS) (Mann, 1999). Surfactants are usually kinds of organic compounds with hydrophobic (tail) and hydrophilic (head). This property causes them to be combined both with water and organic solvent (Mouton et al. 2003).

Surfactants are dual compounds that increase free energy of system by substitution of large molecules with high energy. Due to their high potential in transition of contaminations, surfactants are utilized in soil washing. Currently, application of surfactants has been widely used in industries (Evers et al. 1994; Holmberg 2002; Mulligen 2005). In industrial applications most of surfactants, which have neutral pH based on their charge upon their dissolution in water, are classified and this classification includes anionic, cationic, non-ionic and amphoteric surfactants (Mulligen et al. 2001). Accordingly, a non-ionic surfactant is the base of saponin bio-surfactant. In fact, the surfactants causes removal of oil compounds from soil in two mechanisms including moving of oil particles (excitation of particles) and dissolution of oil compounds in water (Bordoloi and Konwar 2009).

Surfactant acts in boundary layer between solution and pollutant. The hydrophobic part tends to pollutant and is connected to it and hydrophilic part is linked to hydrogen unit and separates pollutant from contamination surface and thus removes the pollutant. Of course, efficiency of surfactant depends on factors such as time, temperature, and concentration as well. The synthetic surfactants negatively affect biological activities, ecosystem, and our surrounding ambience. Although the effect of various surfactants is different, the negative effect and toxicity of these materials is obvious on living cells. The insoluble materials in water are mounted on these surfactants and moved toward vital organs of body. Similarly, these

surfactants cause wide dissemination of them in the environment and contamination of environment (e.g. rivers and seas). Therefore one of the suitable alternatives for synthetic surfactants is to use bio-surfactants which have properties similar to bio-surfactants and at the same time their hazardous effects are fewer (Urum et al. 2003).

The present research aims to analyze and assess efficiency of saponin as bio-surfactant in removal of nickel and vanadium from smeared soil by petroleum and determined according to properties of soils within limit of Shahid Chamran oil pump Station.

2- Materials and Methods

2-1 Area Description and Research Steps

The limit of Shahid Chamran oil pump Station located in Ahwaz oil pumping unit was selected as the studied zone in this investigation. The Ahwaz oil pumping unit is one of the 10 oil pumping units in Khuzestan province that is located at the western side of this city and it is assumed as one of the important centers of Iranian oil pipelines and telecommunications Company to feed Kermanshah, Arak, Tehran and Tabriz Refineries.

After conducting primary studies for determination of pilot test conditions, initially properties of soil in the given zone were examined thereby to simulate these properties under laboratory conditions. Therefore, 5 sampling stations were selected and sampling process was done. The specifications and the map for geographic situation of studied zone

and sampling stations in this survey are determined in Table 1 and Fig1.

At first step, 4 samples of contaminated soil were analyzed in the studied zone with 1 control sample. The concentration of nickel and vanadium, Total Petroleum Hydrocarbons (TPH), and pH were some properties which were measured. The soil texture was also one of important parameters that were measured in terms of percent of sand, clay, and silt. The soil texture was also determined by means of soil texture triangle. One-Way ANOVA as a statistical test was utilized to determine significance level of difference between data in various sampling stations. Using Peterson grab sampler (dimensions: 5×10×15cm), 4 samples were taken from fully smeared soil with petroleum and 1 non-contaminated soil sample was assumed as control one to execute all tests under controlled conditions in vitro to ensure from precision of results in experiments.

Table 1: Geographical coordinates of sampling stations

Location	Geographical coordinates
Station no 1	N32 27 28.7 E48 29 38.7
Station no 2	N32 15 35.8 E48 49 58.6
Station no 3	N32 03 07.1 E48 51 40.4
Station no 4	N31 39 00.0 E48 52 37.0
Station no 5	N31 38 03.4 E48 53 45.0



Fig 1: sampling location

After simulation of soil properties of the

region in vitro, 18 modes were simulated to conduct this test. The effect of three variables of

temperature, pH, and concentration of oil compounds was evaluated on rate of efficiency for soil-washing by extracted saponin solution from leaf of lotus tree. Also Pearson's correlation formula was utilized to determine rate of effect by each of independent variables (temperature, pH, and concentration of oil compounds) on dependent variables (efficiency of soil-washing) where findings showed that pH factor plays an important role in rate of efficiency of soil-washing.

After determination of all modes of soil-washing efficiency, Mamdani fuzzy-type inference system for quantization of modes between the 18 laboratory phases. To this end, at first step 3 input indices including variable of temperature (3 classes), variable of pH (3 classes), and variable of concentration of oil compounds (2 classes) and 1 output variable including rate of soil washing efficiency was defined. Triangular functions were used for determination of degree of membership. In inference part, 18 fuzzy rules were defined and finally in defuzzification unit, mapping of membership degree was defuzzified using centroid technique.

2-2 Preparation and Chemical Digestion of Soil Samples

The leaf of lotus tree was utilized to prepare saponin solution in this study. It was used because of existing wide flora of this tree within area of Khuzestan province. The extraction process following to filtration of saponins was done based on method proposed by Aghel et al. (2007). The Crude oil with density of 812g/L was utilized to smear soil and the sample was prepared directly from Abadan refinery. The soil column was smeared up to 10000 and 30000ppm by Crude oil.

The pilot testing sample of soil-washing was made of 2 Plexiglas columns (height: 60cm- diameter: 9cm) in which a valve was embedded for discharging of washing solution at the end and bottom part for any column. Two containers were also embedded to be placed in washing site each of which was connected to one of both columns. A mesh with aperture size of 0.075mm in circular form was placed at section of column with same size of the diameter of the smallest soil particles placed at the end of column. After pouring of given soil in column, the soil was smeared by petroleum at certain quantity where TPH values were

10000ppm and 30000ppm in this investigation.

The smearing was in such a way that the needed quantity of petroleum was calculated for smearing of soil based on volumetric quantity and then with respect to density, the needed amount of petroleum was measured for smearing of soil in grams by scales and it was mixed with the necessary amount of soil inside column. After smearing, soil column was left for 3 days (72h) to have adequate opportunity for mixture and execution of reactions among soil and petroleum and washing operation was done after 3 days.

After the end of testing process, full digestion process was done to determine concentration of heavy elements (nickel and vanadium). This process was conducted in 18 phases by considering concentration of petroleum in two density rates of 10000ppm (nickel: 235ppm; vanadium: 268ppm) and 30000ppm (nickel: 724ppm, vanadium: 762ppm), at temperatures 15°C, 25°C, 35°C and in equivalent pH levels 7, 6, and 8.

2-3 Fuzzification Of Soil-washing Process

This study determined rate of efficiency of soil-washing of saponin by piloting of 18 laboratory modes in 2 concentrations, 3 temperatures, and 3 different pH. To operationalize this process under natural conditions, the interstitial modes of these 18 laboratory phases should be also determined approximately. In fact, under natural conditions, there is very low possibility for determination of quality of soil washing efficiency by laboratory phases. Thus, a method should be utilized to determine quality of soil-washing efficiency by saponin under given inter-phase laboratory conditions. The fuzzy logic is one of the best solutions for this problem.

2-4 Fuzzification

Selection of membership functions with high efficiency is assumed as the main requisite in designing of a fuzzy expert system for lingual variables and definition of input fuzzy sets. The input indices are converted into fuzzy variables by determination of classes of indices at any group. The resulting function is called membership function for inputs. As a result, membership degree can be determined in this set at any point by defining of membership degree. In fact, membership degree at any point is a mapping of that point in a fuzzy set within

specified interval (between zero and one) and based on form of the defined membership function. Consequently, the rate of ambiguity of membership for a value is expressed quantitatively (Vadiati et al. 2013).

Several types of membership functions have been employed in studies out of which one can refer to triangular, trapezoidal, Gaussian,

and sigmoid functions. Triangular function was selected in this study and based on type of fuzzification.

You can see input and output indices in fuzzy system at this study in the following table. (Table 2)

Table 2: Fuzzy indices, classes and their fuzzy equivalents

indices	Index	Class	Fuzzy equivalent	Mappings data
	pH	6-7	Low	6-7
		7-8	Medium	7-8
		8-9	High	8-9
Input indices	Temperature (°C)	10-20	Low	10-20
		20-30	Medium	20-30
		30-40	High	30-40
	Concentration (ppm)	10000	Low	10000
		30000	High	20000
Output indices	Soil-washing efficiency	18-24%	Very weak	0-0.2
		24-30%	Weak	0.2-0.4
		30-36%	Medium	0.4-0.6
		36-42%	Good	0.6-0.8
		More than 42%	Very good	0.8-1

Numbers of rules needed for fuzzy inference system depend on numbers of classes for any index and it is calculated by the following formula.

$$I=K1 \times K2 \times \dots \times Kn \quad (\text{Eq. 1})$$

Where, in this formula symbol (I) denotes number of rules, (n) is number of indices, and (k) is number of classes. The temperature index and pH indicator both have three classes and the oil concentration index has 2 classes, therefore, the number of fuzzy rules is: $3 * 3 * 2$. This equals to 18 rules.

2- 5 Defuzzification

The fuzzy output is the final result for inference process. It is necessary to convert output from fuzzy state into absolute value for

practical use and possibility for using it in assessment of soil-washing efficiency and decision- making. This part of inference process is well-known as defuzzification. In fact, it is a unit which has operated as a function of a fuzzy set to an absolute value. Various and numerous techniques have been also developed for defuzzification of fuzzy output in inference process such as centroid method, center of sums, height, center of greatest area, and mean maximum where in this study centroid method was employed (Hashemi et al. 2010).

3. Results and Discussion

The results of measuring the soil properties are shown in Tables 3.

Table 3: The resultant findings from calibration of heavy metals, pH, and total petroleum hydrocarbons (TPHs)

	Concentration of nickel (ppm)	Concentration of vanadium (ppm)	TPHs (ppm)	pH
First sample	286.2	361.2	657	7.04
Second sample	282.1	289.3	601	7.35
Third sample	279.0	306.5	614	7.23
Fourth sample	282.6	223.8	599	7.34
Control sample	131.3	185.4	219	6.58
Range	154.90	137.40	438	0.77
Mean	252.24	293.24	538	7.108
Standard deviation	67.65	49.22	179.85	0.3204
Variance	4577.30	2423.32	3234	0.101
Kurtosis coefficient	-2.228	-0.067	-2.139	-1.508
Skewness coefficient	4.972	1.497	4.686	1.995

You can see states of assessment of efficiency rate in the 18 measurement modes in Tables 4 and 6. You observe rate of soil-

washing efficiency in 18 measured modes in figure 1 and 2.

Table 4: The modes of assessment in measurement of efficiency at 9 modes with concentration 10000ppm

	Pilot phases	Petroleum concentration (ppm)	Concentration of nickel (ppm)	Concentration of vanadium (ppm)	pH	Temperature
First sample	Phase 1	10000	235	268	6	15
	Phase 2	10000	235	268	6	25
	Phase 3	10000	235	268	6	35
	Phase 4	10000	235	268	7	15
	Phase 5	10000	235	268	7	25
	Phase 6	10000	235	268	7	35
	Phase 7	10000	235	268	8	15
	Phase 8	10000	235	268	8	25
	Phase 9	10000	235	268	8	35

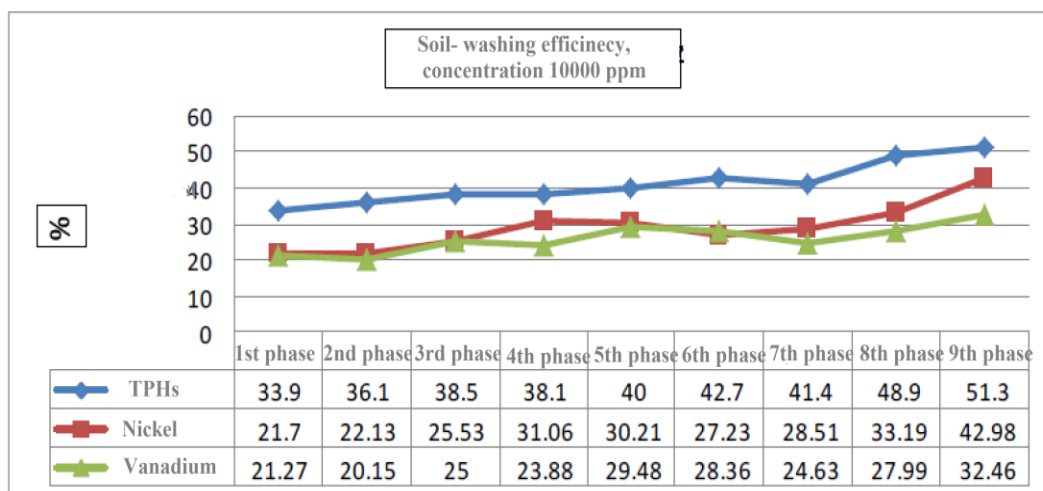


Fig 1: Soil-washing efficiency for nickel and vanadium at 9 pilot modes with concentration of 10000ppm

Table 5: Correlation coefficient for independent variables in soil-washing efficiency at pilot phase (10000ppm)

		pH	TPHs	Temperature
Nickel	Correlation coefficient	-0.78 *	-	-0.32
	Sig- value	0.12	-	0.39
Vanadium	Correlation coefficient	-0.67 *	-	-0.58
	Sig- value	0.04	-	0.101
TPHs	Correlation coefficient	-	-	-
	Sig- value	-	-	-

Table 5 has shown that correlation coefficient of pH is inversely related to soil-washing efficiency (-0.789). Namely, concentration has been reduced in nickel and

vanadium after soil-washing as pH value increased. This correlation coefficient was also calculated for variable of temperature -0.323 that indicates lower correlation than pH.

Table 6: The modes of assessment in measurement of efficiency at 9 modes with concentration 30000ppm

	Pilot phases	Petroleum concentration (ppm)	Concentration of nickel (ppm)	Concentration of vanadium (ppm)	pH	Temperature
First sample	Phase 10	30000	724	752	6	15
	Phase 11	30000	724	752	6	25
	Phase 12	30000	724	752	6	35
	Phase 13	30000	724	752	7	15
	Phase 14	30000	724	752	7	25
	Phase 15	30000	724	752	7	35
	Phase 16	30000	724	752	8	15
	Phase 17	30000	724	752	8	25
	Phase 18	30000	724	752	8	35

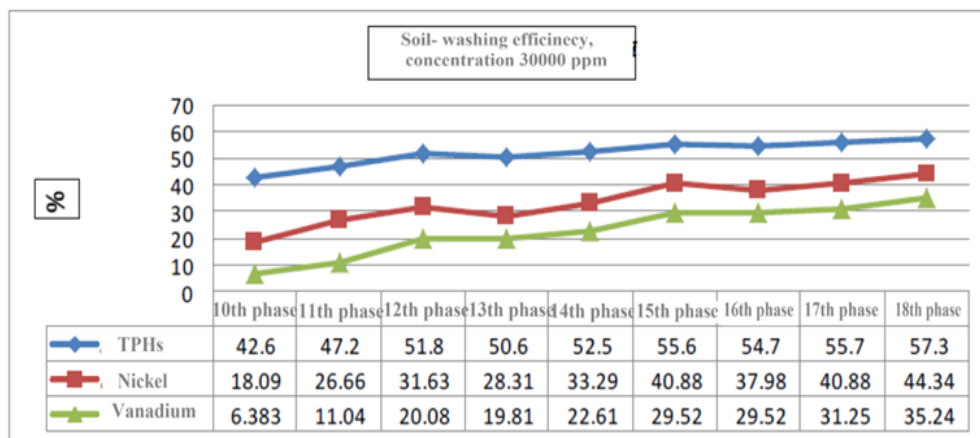


Fig 2: Soil-washing efficiency for nickel and vanadium at 9 pilot modes with concentration of 30000ppm

Table 7: Correlation coefficient for independent variables in soil-washing efficiency at pilot phase (30000ppm)

		pH	TPHs	Temperature
Nickel	Correlation coefficient	-0.878	-	-0.437
	Sig- value	0.002	-	0.240
Vanadium	Correlation coefficient	-0.807	-	-0.559
	Sig- value	0.009	-	0.117
TPHs	Correlation coefficient	-	-	-
	Sig- value	-	-	-

In nickel element, Pearson's correlation coefficient shows high negative relationship among soil-washing and this variable for variable of pH (-0.878) and sig-value (> 0.002). This value was calculated -0.787 for vanadium. The variable of temperature was computed - 0.437 for nickel and -0.550 for vanadium. The

existing relationship among rise of temperature and pH with reduced concentration of nickel and vanadium after soil-washing by saponin is an important point. Namely, soil-washing efficiency increased by rise of temperature and pH.

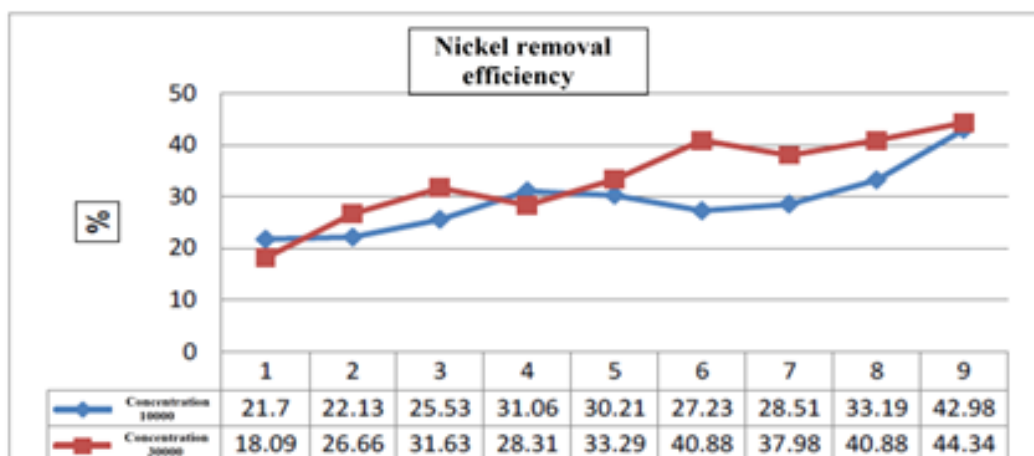


Fig 3: Comparison of soil-washing efficiency in removal of nickel in both measured concentrations

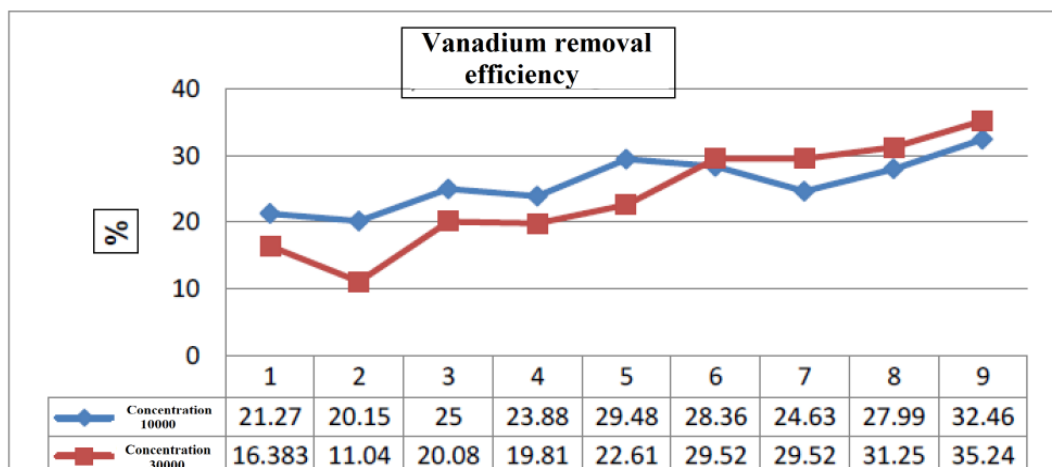


Fig 4: Comparison of soil-washing efficiency in removal of vanadium in both measured concentrations

Table 8: Correlation coefficient of independent variables in soil- washing efficiency in accumulation of 18 measured pilot modes

		pH	TPHs	Temperature
Nickel	Correlation coefficient	-0.767	0.601	-0.28
	Sig- value	0.107	0.008	0.75
Vanadium	Correlation coefficient	-0.580	0.572	-0.12
	Sig- value	0.075	0.013	0.611
TPHs	Correlation coefficient	-0.167	0.601	-0.08
	Sig- value	0.507	0.008	0.75

The given results from assessment of efficiency rate for removal of heavy metals showed that the best efficiency for removal of nickel and vanadium was obtained at temperature 35°C and pH about 9. Under concentration 10000ppm, efficiency for removal of nickel was shown 42.98% in constrained mode and 32.46% for efficiency if removal of vanadium. Under concentration 30000ppm, rate of efficiency for removal of nickel and vanadium was 44.34% and 35.24% respectively and this indicates efficiency of soil-washing rises by increase in concentration of oil compounds.

You can also see the given results from

Pearson’s correlation test for assessment of effect of variables of temperature, concentration, and pH in Table 8. The correlation coefficient was -0.76 and -0.580 for variables of pH to remove nickel and vanadium. This indicates that as the pH rises, the efficiency of soil washing increases. The correlation coefficient of temperature was determined -0.281 and -0.129 for nickel and vanadium respectively which shows lesser correlation than pH.

The correlation coefficient was negative in all cases and it showed rise of temperature, pH, and concentration of nickel and vanadium reduced after soil-washing.

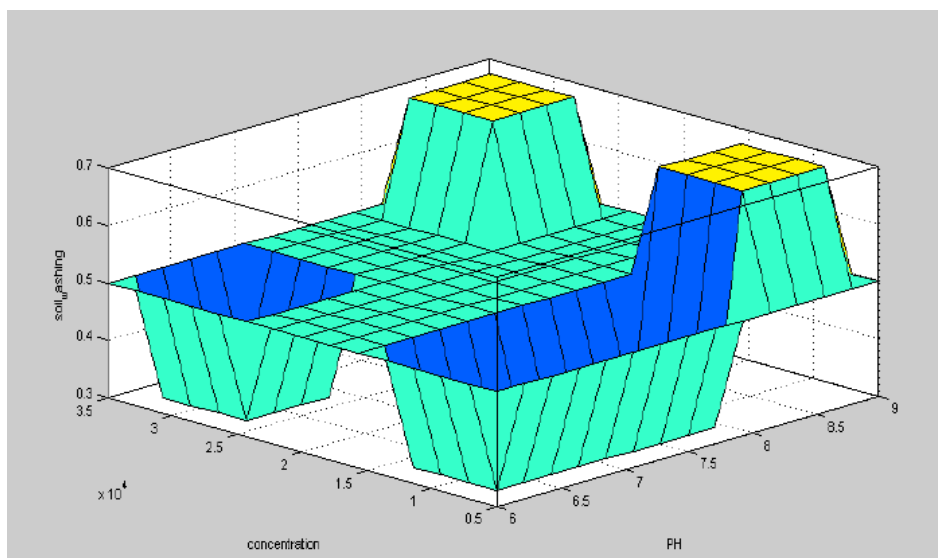


Fig 5: The created fuzzy relationship between input and output indices

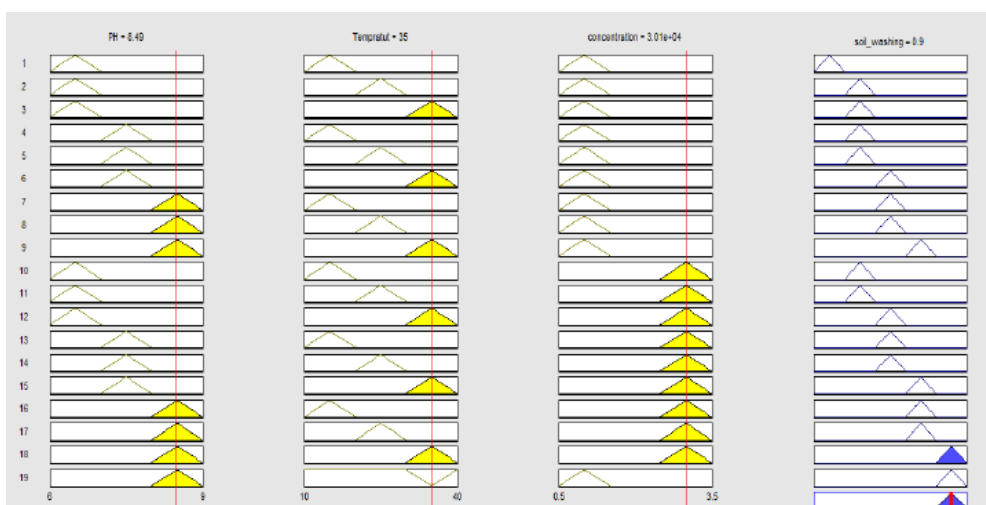


Fig 6: Non-fuzzy mapping of soil-washing efficiency against change in any independent variable

As it shown in Fig 3, by change in input level of any index, the given mapping is visible in output index. In base mode, the most ideal state for soil-washing by saponin includes pH (about 9), temperature (35°C), and concentration 30000ppm. The relative efficiency may be determined by means of fuzzy system under normal conditions. since this research has been conducted only to assessment of efficiency of saponin in the soil-washing and it is not intended for ranking of managerial scenarios and managerial decisions for implementation under non- laboratory conditions, fuzzy inference system is stopped at

this level.

4- Conclusion

The efficiency of saponin as bio-surfactant was examined in removal of heavy metals e.g. nickel and vanadium which were assumed as oil pollution index based on properties of soils within limits of Shahid Chamran oil Pump Station in 2016. To this end, in order to determine pilot condition of test after conducting of initial studies, properties of soil of this region should be examined firstly. Five stations were sampled. Parameters of nickel and vanadium, total petroleum hydrocarbons, and pH were measured. Inductively coupled plasma

technique was used for calibration of heavy metals. Total petroleum hydrocarbons were calibrated with GC technique and pH was measured by pH-meter.

The mean concentration of nickel was estimated about 281ppb within studied zone. Similarly, the mean concentration of vanadium was also determined about 339 ppb. TPHs were determined about 600 ppb and also pH within range (6.5-8).

Likewise, soil texture of this region was also measured where based on the given results, percent of sand, clay and silt, soil type in triangle of soil texture, and silty density were determined. The findings generally showed that the quantities of nickel, vanadium, total petroleum hydrocarbons (TPHs), and pH in the contaminated areas are different from control sample.

After determination of soil properties in the studied zone, rate of soil-washing efficiency was measured in 18 laboratory phases. The studied phases include 3 variables of temperature within ranges (15°C, 25°C, 35°C), pH within range of 6, 7, and 8, and total petroleum hydrocarbons (TPHs) within range 10000ppm and 30000ppm. The leaf of lotus tree, which grows in tropical regions, was used for preparation saponin solution. Aghel technique was adapted for extraction of saponin. The Crude oil with density of 812g/L was prepared from Abadan refinery and used for smearing of soil with the studied concentrations.

The findings resulting from assessment of rate of efficiency in removal of heavy metals showed that the best removal efficiency was obtained for nickel and vanadium at temperature (35°C) and pH (about 9). The rate of removal efficiency was shown 42.98% under concentration 10000ppm and 32.46% for removal of vanadium. The rate of removal efficiency for nickel and vanadium was respectively 44.34% and 35.24% under concentration 30000ppm which showed efficiency of soil-washing rose following the increase in concentration of oil compounds.

The correlation coefficient was derived for variable of pH to remove nickel (-0.767) and vanadium (-0.580) respectively. This shows rise of soil-washing efficiency at high pH. The

correlation coefficient in temperature was determined -0.281 for nickel and -0.129 for vanadium where this showed smaller correlation rate than pH.

The correlation coefficient was negative in all cases that indicated the concentration of nickel and cadmium was reduced after soil-washing as temperature and pH increased.

Fuzzy inference system and MATLAB software were utilized for fuzzification of interstitial modes between laboratory scenarios. For this purpose, 3 input indices were defined after determination of initial fuzzy system including temperature, pH, and concentration of oil compounds with an output index i.e. soil-washing efficiency. The indices were accurately defined according to laboratory conditions. Triangular functions were utilized for determination of membership functions. In inference unit, 18 fuzzy rules were made. At the last step, defuzzification was executed and mapping was determined for all modes to interstitial conditions between variables that might play important role.

Concentration of elements of nickel and vanadium was examined in soils within oil zone and it was measured in 5 stations including 4 contaminated stations and a zone as control sample and they were given in Table 4-1. The results showed the concentration of these studied compounds significantly differed from control zone (Sig=0.03).

The results of study indicated that rate of efficiency was different under various conditions of temperature, pH, and rate of contamination with oil compounds. The best efficiency of removal for nickel and vanadium was obtained at temperature 35°C and pH level (about 9). The correlation coefficient for removal of nickel and vanadium to pH variable was -0.281 and -0.129 respectively that indicated rise of soil-washing efficiency at high pH. The correlation coefficient of temperature was determined -0.281 and -0.129 for nickel and vanadium respectively which indicated smaller correlation than pH. The correlation coefficient was negative in all cases that showed concentration of nickel and vanadium was reduced after soil-washing by rise of temperature and pH.

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