Water Quality Index of Sungai Tunggak: An Analytical Study

Nasly M. A., M. A. Hossain and Mir Sujaul Islam

Abstract**—** Water quality deterioration of Tunggak River is the impact of rapid industrialization at Gebeng, Malaysia. The aim of the study was to evaluate the water quality of the river using the application of WQI. To achieve the objectives 180 water samples were collected and comprehensive physicochemical analysis was done using APHA & HACH standard methods of analysis. The WQI was calculated using DOE-WQI based on the concentration of DO, BOD, COD, SS, pH and NH3-N. Results showed the sequence of monitoring stations 7<5<3<2<6<4<8<10<1<9 based on WQI value; where the first 7 (mid-stream) stations were categorized as class IV (highly polluted) and the last 3 were classified as class III (polluted). It was mainly because of low concentration of DO and high concentration of BOD, COD and NH3-N in the mid-stream due to the industrial activities. According to the INWQS, Malaysia water of the river cannot be used except irrigation.

Keywords**—**Ammoniacal Nitrogen (NH_3-N), Bio-chemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Water quality index.

I. Introduction

WATER is the essential part of environment and living being. The earth is like a water planet; as 71% of the earth is covered with water [1]. But, fresh water which is only 2.5% of all water is a scarce resource of the earth and again the water which is available for use is only 0.4% of total fresh water as like as tea spoon that represent the available water [2]. This little water source is also polluting throughout the world. Water quality deterioration is the common problem nowadays. It is the main factor that controls the health and disease in both man and animals [3]. River water as well as surface water quality is largely depends on the natural processes and anthropogenic activities like municipal, homesteads & agricultural and industrial wastewater discharges; which constitute a continuous polluting source [4],[5]. Speedy population growth and the accelerated pace of industrialization in the last few decades' increased tremendous pressure on the demand of fresh water [6].

Malaysia is rich with its bounty of water resources. It is contributing to the economic and industrial development of the country [7]. At the same time Malaysia is the nation that faced the serious water crisis in 1998 [8]. The present situation is also changing day by day with population growth, urbanization and industrialization. According to the Environmental Quality Report 2010, 50% river water of Malaysia is polluted which is higher than previous couple of years [9]. Industries are generating conventional and non-conventional pollutant and discharging into the river flow that causes the deterioration of water quality [10].

Gebeng is the main industrial park of Pahang Malaysia; where Tunggak is a strategic important river. Rapid industrialization at Gebeng producing lots of effluents and they are discharging those effluents into the river Tunggak; as result polluting the water of the river. Therefore, the objective of the study is to provide information on the physico-chemical properties of the river water and to evaluate the water quality status of the river with DOE-WQI.

II. Materials and Methods

A. Study area and monitoring stations

The geographical location of Gebeng is 3° 55' 0" N to 4° 01' 0" N and 103° 22' 0"E to 103° 27' 0"E; and, the adjacent river Tunggak is situated in between 3056′06″ to 3059′44″N and 103022′42″ to 103024′47″E (Fig. 1). Gebeng industrial estate is consist of multifarious industries like metal, chemical, petrochemical, polypropylene, polymer, palm oil, food, mining, wooden and gas & power industries. Tunggak is the most important river that carries maximum wastes from the area. The Tunggak River originated at the uphill of Gebeng area. At near Angler marine centre it joined with another river namely Balok and ultimately flows into South China Sea [10]. Considering the land use pattern, point sources of pollution and river network 10 monitoring station was selected for sampling using GPS (Fig. 1).

B. Sampling and in-situ data collection

Water samples were collected from 10 monitoring station monthly for 6 months from February 2012 to July 2012. A total of 180 river water samples were collected. At the same time some in-situ data of temperature, pH, conductivity, turbidity, TDS, DO and salinity were collected by using YSI. During sampling, transportation and preservation of water samples APHA and HACH standard procedures were followed [11], [12]. For BOD sample separate sampling bottles were used.

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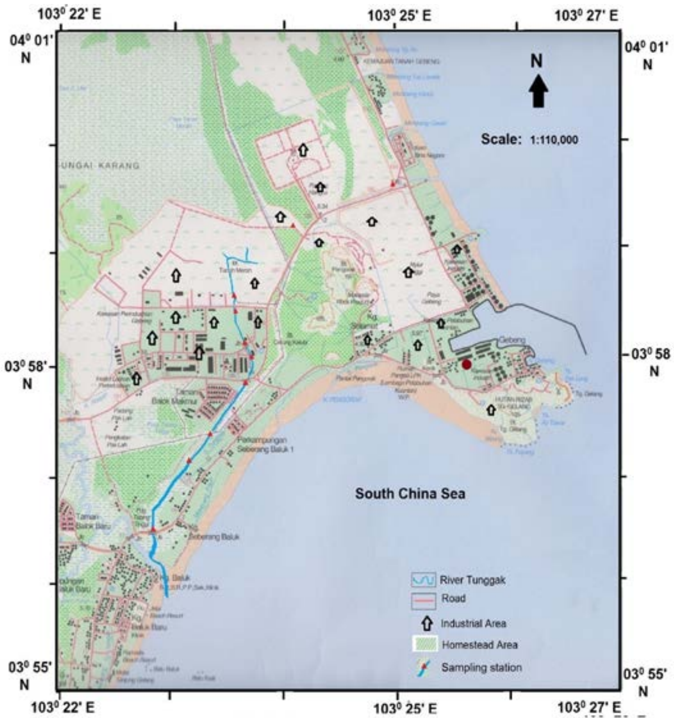

Fig. 3 Location map of the study area indication monitoring stations

C. Laboratory analysis

After collecting, the samples were analyzed in laboratory for determining the concentration of ammoniacal nitrogen (NH3-N) in nessler method, nitrate nitrogen (NO3-N) in cadmium reduction method, phosphorus (PO43-) in ascorbic acid method, and sulfur (SO4) in sulfavar 4 methods. COD was analyzed in reactor digestion method and regarding BOD, the first reading was taken just after collecting the samples and was preserved in incubator at 20^0C temperature for 5 days. After 5 days final reading was taken and BOD was calculated with the formulae: BOD (mg/L) = (DO_i – DO_5)/ P; where, DOi = DO (mg/L) of diluted sample about 15 minutes after preparation, DO_5 = DO (mg/L) of diluted sample after 5 days incubation at 200C and P = decimal volumetric fraction of sample [11]. Heavy metals were determined by using ICP-MS.

D.Statistical analysis

The statistical analysis was done for correlation with SPSS 16.0 software. Water quality index was calculated by using DOE-WQI.

E. Water Quality Index

The comparison of water quality parameters with their respective regulatory standards is the basis of water quality index [13]. The water quality index was calculated based on the concentration of DO, BOD, COD, ammoniacal-N, SS and pH of the study area [14],[15]. By estimating the values of sub-index of above parameters the water quality index was obtained. Following formula was used to calculate the WQI.

WQI= 0.22*SIDO + 0.19*SIBOD + 0.16*SICOD + 0.15*SIAN + 0.16*SISS + 0.12*SIPH (* denote multiplication); where the sub-indices of those parameters were obtained from a series of equations.

III. Results and discussion

The pH which is an important indicator for the water quality [3] varied between 4.23 and 9.12 as shown in the Table I. The mean pH value was 6.77. The pH at upper stream was found to be acidic compare to the middle and lower stream where alkaline pH was observed. The lower stream of the river is joined with South China Sea and the tidal interference was presence there; which might be the reason of higher pH as average pH of sea water is 7.5-8.4[16]. At the upper stream received wastes from some industries which were rich in acidic substances and at the middle the reason was obviously the temperature and industrial wastes [10]. The highest Turbidity values were obtained at station 5 where the maximum turbidity recorded 200 NTU (Table I) and lowest was at station 8. The overall mean turbidity value was observed 15.59 which were above the Malaysian standard [17]. It is mentionable that the maximum concentration was recorded in the mid-region where more industrial activities were persisting. Regarding TDS the concentration ranged between 7.7- 49400 mg/L where the highest value was observed at the lower stream of the river. It was perhaps due to the presence of tidal influence, forested area and homestead activities in those areas; again, TDS was found positively highly correlated with TSS (Table II), which was also higher in lower stream (Table I). However, the overall mean of TDS was recorded 4735.23 mg/L which was beyond the Malaysian standard limit [17]. High concentration of TDS in surface water is the indicator of intense anthropogenic activities along the river area [3]. Total suspended solids were found in the range of 2-75 mg/L with an overall average 16.21 mg/L which was within the standard limit of Malaysia. But in some part of the river the values found beyond the limit; such as, the lower stream and one of middle station (Table I). This was perhaps because of tidal interference and industrial effluents.

Dissolved oxygen (DO) which is the most important parameter for water quality was determined and concentration was recorded. At the whole area DO concentration were found less than the standard limit of Malaysia [17]. The average value was recorded 2.24 mg/L; which indicated that the water of the area was highly deoxygenated. It was due to the discharge of industrial wastes containing high concentration of organic matter and nutrient [3] and probably due to the microbial activities to degrade the organic matter [18].

Nitrogen concentration was determined in the form of NH3-N and NO3-N. NH3-N is an important parameter for water quality analysis; as water quality degradation due to ammoniacal nitrogen remains a crucial environmental and public concern worldwide; because it can cause eutrophication [19]. In the present study it was found that almost all the part of the river water contained higher concentration of ammoniacal nitrogen compare to the national standard of Malaysia [17]. High concentration of NH3-N was due to industrial activities, especially chemical and petrochemical industries in the area [19]. Nitrate is naturally an ion that is undesirable in water. It can cause methaemoglobinaemia in infants [20]; however, the nitrate value varies from 0-4.5 mg/L with an average 0.12 mg/L. The highest concentration was recorded at station 5 followed by station 6 & 4 and the lowest was at station 9 (Table I). It was because of intense industrial activities at that region and higher pH values; as pH was found positively highly correlated (Table II). The phosphate values obtained higher almost in all stations except station 7 -9 compare to the standard limit of Malaysia [17]; due to industrial effluents and positive correlation with turbidity might be the cause of higher concentration (Table II). The minimum and maximum values were 0.001mg/L and 37.2 mg/L respectively (Table I) with an overall average concentration 1.71 mg/L; which indicated the presence of pollution in water due to industrial effluents and domestic waste-water. Sulphate concentration was determined and result showed that the concentration was within the Malaysian standard except station 7 and 1 (Table I). It might be due to station 1 received sea water containing higher level of SO_4 [21] and station7 is adjacent with some industries that produce detergent and discharge sulfur rich effluents into the river. At the same time SO_4 was found highly correlated with TDS (Table II); at these two stations TDS concentration was also higher.

TABLE I
MEAN, MAXIMUM & MINIMUM VALUES AND STANDARD DEVIATION OF WATER QUALITY PARAMETERS OF SUNGAI TUNGGAK

Station	Statistical tools	DO	BOD	COD	SS	NH_3-N	NO_3-N	SO_4	PO_4^{3-}	TDS	pH	Turbidity	Cr	Co
		%	mg/L									NTU	ppm	
1.	Mean	2.13	7.97	31.23	30.67	1.70	0.12	639	0.63	29585	6.39	14.1	0.01	0.09
	Max	3.31	12.85	47.00	75.00	2.46	0.23	1220	1.15	49400	7.02	22.5	0.02	0.12
	Min	1.29	2.90	19.10	6.00	0.65	0.02	210	0.06	9041	5.66	7.7	0.00	0.06
	STDEV	0.61	3.44	10.29	20.86	0.67	0.08	332	0.38	15160	0.42	5.4	0.01	0.02
2.	Mean	1.56	20.46	45.33	24.33	2.72	0.30	123	1.15	5565	7.38	16.0	0.00	0.15
	Max	2.78	35.90	68.00	72.00	3.90	0.43	320	2.07	7270	7.89	24.7	0.00	0.18
	Min	0.83	6.10	21.00	3.00	2.07	0.10	51	0.59	3110	6.77	9.1	0.00	0.10
	STDEV	0.70	11.37	16.83	20.67	0.57	0.12	117	0.62	1460	0.36	4.8	0.00	0.04
3.	Mean	2.60	24.40	45.08	10.42	2.79	0.32	64.7	1.11	2116.1	7.62	14.2	0.00	0.22
	Max	5.04	37.20	72.00	20.00	4.05	0.66	140	1.74	4720	8.40	22.5	0.00	0.26
	Min	1.00	8.45	13.00	4.00	0.98	0.00	20	0.29	650	7.32	8.6	0.00	0.15
	STDEV	1.52	10.78	20.85	5.33	1.10	0.22	41.7	0.47	1592.3	0.28	4.1	0.00	0.04
4.	Mean	3.34	23.85	46.92	10.08	1.94	0.79	94.2	0.62	2237.3	7.81	12.0	0.01	0.25
	Max	5.34	37.50	68.00	21.00	3.25	2.70	260	1.20	5320	8.51	17.3	0.01	0.35
	Min	1.22	9.05	21.00	3.00	1.08	0.01	20	0.29	527	7.44	8.4	0.00	0.13
	STDEV	1.52	10.76	16.08	5.37	0.87	0.95	92.1	0.36	1977.8	0.3	3.3	0.00	0.09
5.	Mean	2.49	25.23	54.17	30.50	1.83	1.19	111	0.81	2140.1	7.70	47.2	0.01	0.33
	Max	3.08	36.85	92.00	67.00	3.25	4.50	310	1.54	5170	8.95	200	0.01	0.42
	Min	1.45	9.80	23.00	12.00	0.73	0.00	37	0.24	642	6.96	8.9	0.01	0.28
	STDEV	0.58	9.98	21.67	21.42	0.88	1.77	114	0.54	1798.9	0.72	57.9	0.00	0.05
6.	Mean	1.63	26.51	55.83	14.17	1.59	1.37	133	0.58	3026.4	7.81	16.9	0.00	0.74
	Max	2.38	35.50	89.00	24.00	3.35	3.70	430	1.03	7610	9.12	28.8	0.00	0.89
	Min	1.17	7.75	32.00	2.00	0.31	0.00	17	0.15	649	7.25	9.0	0.00	0.64
	STDEV	0.46	11.02	20.38	5.97	1.19	1.10	175	0.33	2908.9	0.59	7.6	0.00	0.09
7.	Mean	3.27	35.48	95.50	12.25	1.50	0.14	366	0.08	1084.3	7.38	8.6	0.04	0.01
	Max	4.51	38.35	116.00	19.00	1.76	0.42	680	0.10	2290	8.6	12.4	0.05	0.02
	Min	2.11	32.30	62.00	8.00	0.86	0.00	170	0.05	203	6.65	6.0	0.03	0.00
	STDEV	0.93	2.75	18.25	3.60	0.34	0.17	194	0.02	831.5	0.57	2.4	0.01	0.01
8.	Mean	2.12	12.4	35.25	11.42	1.65	0.00	14.1	0.03	177.4	4.97	9.2	0.03	0.01
	Max	3.19	22.90	78.00	17.00	2.27	0.01	51	0.06	615	5.42	20.6	0.04	0.02
	Min	1.44	1.35	22.00	5.00	0.96	0.00	0.00	0.01	19.6	4.66	4.6	0.02	0.00
	STDEV	0.55	7.49	17.93	3.65	0.36	0.00	19.8	0.01	232.6	0.29	6.0	0.01	0.01
9.	Mean	1.45	5.88	22.08	9.42	0.25	0.00	1.00	0.04	25.5	5.05	6.9	0.03	0.05
	Max	2.29	10.55	79.00	36.00	0.60	0.00	3.00	0.10	48.7	6.70	22.6	0.04	0.22
	Min	0.76	0.10	2.00	2.00	0.00	0.00	0.00	0.01	7.7	4.23	1.6	0.02	0.01
	STDEV	0.45	3.66	23.62	8.99	0.17	0.00	1.21	0.03	18.2	0.96	7.5	0.01	0.08
10.	Mean	1.80	10.67	14.08	8.83	2.40	0.19	42.8	12.1	1395.5	5.58	10.9	0.04	0.02
	Max	2.26	18.45	41.00	15.00	3.40	0.28	69	37.2	2930	6.4	30.2	0.06	0.02
	Min	0.79	2.20	7.00	2.00	1.80	0.09	30	0.07	333	4.92	6.7	0.02	0.00
	STDEV	0.39	6.87	10.05	3.97	0.56	0.07	10.9	15.0	1102.4	0.45	6.4	0.01	0.01

Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD) were analyzed and results were shown in Table I. The range of COD values was 2.0- 116.0 mg/L and overall mean was 45.55 mg/L. The highest value was recorded at station 7 and the lowest was at station 10. It revealed that COD was found to be higher in all station compare to Malaysian standard and it was found maximum at middle region; which indicated the presence of both oxidizable organic and inorganic pollutants that polluting the solids waste [22]. The BOD results were also same as COD. Similar to COD, the highest BOD value was found at station 7; but the lowest was at station 9 (Table I). According to INWQS

Malaysia the water of Tunggak River was found to be in class V based on the concentration of COD and BOD [17].

TABLE II
CORRELATION COEFFICIENT MATRIX OF WATER QUALITY PARAMETERS AT THE STUDY AREA

	pH	DO	BOD	COD	SS	NH3-N	Turbidity	TDS	SO4	NO3	PO4	Cr	Co
pH	1												
DO	0.4770	1											
BOD	0.8206**	0.6207	1										
COD	0.6446*	0.6143	0.9084**	1									
SS	0.2837	-0.1126	-0.0238	0.0738	1								
NH3-N	0.4485	0.1586	0.2912	0.0224	0.1437	1							
Turbidity	0.4480	0.0712	0.2798	0.1596	0.6699*	0.1705	1						
TDS	0.0028	-0.0861	-0.3509	-0.1778	0.6502*	0.0464	-0.0489	1					
SO4	0.1817	0.2289	0.0439	0.2919	0.5627	-0.0351	0.1222	0.8580**	1				
NO3	0.6938*	0.1000	0.5086	0.2872	0.2455	0.1078	0.7872**	-0.1567	-0.1375	1			
PO4	-0.2864	-0.2300	-0.2898	-0.4818	-0.2457	0.3598	0.8018**	-0.1089	-0.2052	-0.1342	1		
Cr	-0.7082*	-0.0301	-0.2998	-0.1316	-0.3977	-0.4263	-0.3255	-0.2676	-0.0960	-0.5545	0.4532	1	
Co	0.6189	-0.1187	0.4111	0.2208	0.1351	0.0577	0.4011	-0.0685	-0.1013	0.9034**	-0.2292	-0.6595*	1

Significant level indicated as * for $p < 0.05$ and ** for $p < 0.01$

Chromium (Cr) and Cobalt (Co) were determined by using ICP-MS spectrometry and results showed in Table I. It was found that Cr was within the permissible limit at all stations while the Co concentration was found to be beyond the standard limit [23]. Table II stated the correlation between parameters where the correlation between Cr and Co were found negative significantly correlated; which indicated that due to less Cr at the river water Co concentration was higher. However, Co concentration was found to be within the normal limit at station 1 and 9.

Water quality index

For the water quality index calculation The DOE-WQI values were computed according to the procedure stated at methodology. To estimate the sub-index values the best fit equations were used (Table III). Based on the sub-index values water quality classification was done and demonstrated at Table IV. Table IV showed that the lowest WQI was found at station 7 with 37.07 followed by station 5 (40.46) and station 3 (41.08). The sequence of water quality deterioration based on WQI was found to be 7>5>3>2>6>4>8>10>1>9. It stated that the surface water quality of the river was found polluted at all parts. At the mid-stream (station 2 -8) it was more and the water of those stations was categorized as class IV(highly polluted); the lowest and uppermost stations were classified in class III (polluted). As can be seen the mid-stream region of the river was found to be more polluted compare to the lower and upper stream. It was the indication of higher anthropogenic activities at that part. Actually, most of the chemical, petrochemical, metal, wooden, gas & power, mining and food industries were situated in the mid-stream region. As a result they were producing wastes and discharged untreated or partially treated wastes to the river flow which resulted more deterioration of water quality at the mid-stations; and the water of that part (station 2 to 8) were found to be un-usable without irrigation [17]. Only the lowest stream station and uppermost stations were found to be class III, perhaps because of tidal interference at lowest stream and less industrial activities at uppermost stream region. Although the uppermost station were actually the starting part of the river, from where it was originated. The water flow in that area was low and also industrial activities were less there. The water of those stations can be used for water supply only after extensive treatment and for fisheries with tolerant species [17].

TABLE III
BEST FIT EQUATIONS FOR THE ESTIMATION OF THE SUB-INDEX VALUES

Sub-index	WQI Calculation	Ranges
SIDO	= 0	For $x \le 8$
	= 100	For $x \ge 92$
	= - 0.395 + 0.03 x2 – 0.0002 x3	For 8 < x < 92
SIBOD	= 100.4 – 4.23 x	For $x \le 5$
	= 108 e0.055x – 0.1 x	For x > 5
SICOD	= - 1.33 x + 99.1	For $x \le 20$
	= 103 e0.0157x – 0.04 x	For x > 20
SIAN	= 100.5 – 105 x	For $x \le 0.3$
	= 94 e0.573x – 5 | x – 2 |	For 0.3 < x < 4
	= 0	For $x \ge 4$
SISS	= 97.5 e0.00676x +0.05 x	For $x \le 100$
	= 71 e0.0061x – 0.015 x	For 100 < x < 1000
	= 0	For $x \ge 1000$
pH (SIPH)	= 17.2 – 17.2 x + 5.02 x2	For x < 5.5
	= - 242 + 95.5 x – 6.67 x2	For $5.5 \le x < 7$
	= - 181 + 82.4 x – 6.05 x2	For $7 \le x < 8.75$
	= 536 – 77 x + 2.76 x2	For $x \ge 8.75$
WQI	= 0.15 x SIAN + 0.19 x SIBOD + 0.16 x SICOD + 0.22 x SIDO + 0.16 x SISS + 0.12 x SIPH	

TABLE IV
WATER QUALITY CLASSIFICATION OF TUNGGAK RIVER BASED ON DOE-WQI

Station	DOSI	BOD SI	COD SI	AN SI	SS SI	pH SI	WQI	CLASS	WQ STATUS
1	0	69	62	34	81	96	52.51	III	Polluted
2	0	33	49	16	84	98	41.64	IV	Highly Polluted
3	0	26	49	15	91	96	41.08	IV	Highly Polluted
4	0	27	47	31	92	94	43.13	IV	Highly Polluted
5	0	24	42	32	81	95	40.46	IV	Highly Polluted
6	0	22	41	36	89	94	41.65	IV	Highly Polluted
7	0	12	19	37	90	98	37.07	IV	Highly Polluted
8	0	53	58	35	91	56	45.82	IV	Highly Polluted
9	0	78	72	74	92	58	59.10	III	Polluted
10	0	59	80	22	92	83	52.08	III	Polluted

IV. CONCLUSION

The study result revealed that the water of Tunggak River is not suitable for public consumption even with extensive treatment. The water of a little part of the river at the lowest and uppermost part can be used for water supply after extensive treatment and for some selective tolerant species of fish cultivation. However, the water of Tunggak River can be used for only irrigation as per the INWQS Malaysian standards. The study also showed that, application of Water Quality Index (WQI) in assessing the overall quality of river water was helpful and easily understandable. This method of water quality index assessment appears to be more systematic and comparative evaluation of the water quality of different sampling stations was obtained. It is easy for public understanding about the quality of water as well as a useful tool in the field of water quality management in many ways.

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Effects of temperature and salinity on the germination of *Medicago arborea* L.

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***Abstract*—** This study consist to study the effects of salt (NaCl) and temperature on the germination behavior of seeds of *Medicago arborea*. The germination tests were carried out at different temperatures (5 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C). For each temperature, different NaCl concentrations were tested (0.5, 1, 1.5, 2, 3, 4, 8 and 16 g / l). The measurements concerned the capacity, coefficient of velocity, latency and average time of germination.
The results showed that the germination of *Medicago arborea* tolerate NaCl concentrations of 8 g/l. However, in the presence of salt, the germination is optimally between 0.5 and 2 g/l, decreases beyond 2 g/l and vanishes at 16g/l. In the different salt concentration including the control test, the optimum germination temperature is between 20 °C and 25 °C. At these two temperatures, the germination of Medicago arborea is much more resistant with NaCl concentrations up to 8 g/l. This is not the case at low (5 °C) and high temperatures (30 °C, 35 °C, 40 °C) where the tolerance is less important.

***Keywords*—** Germination, *Medicago arborea*, salinity, temperature.

I. Introduction

SPECIES of the genus Medicago have agronomic interest due to their ability to the symbiotic nitrogen fixation, allowing an abundant production of plant proteins. In Algeria, these plants provide improved flora and grazed fallow easily fall in rotation with cereals [1]. In addition, work has been undertaken to use leaf extracts of alfalfa (case of *Medicago sativa*) as food for people suffering from high dietary deficiency, especially in children [2].

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These species can be used in breeding programs of plant material that can be used in projects to improve pastoral care and the fight against desertification. Algeria proposed a project to plant these species, like *Medicago arborea*, in steppe regions for their interest of soil fixation and fight against erosion Sahara. However, the use of such species requires a better understanding of their biology and their mechanisms of regeneration and growth.
In this context, the objectives of our work are to highlight the combined effect of salinity and temperature on seed germination of *Medicago arborea*. It is research the optimum temperature for germination and tolerance to salt (sodium chloride).

II. Biological Material and Methods

A. Biological Material

The present work aims to determine the combined effect of salinity by sodium chloride (NaCl) and temperature on the germination of seeds of *Medicago arborea*. The seeds have been delivered by the National Institute of Forestry Research (INRF) of Ain Skhouna station, located in the southwest of the province of Saida (Algeria). It's were harvested in June 2011 and stored at ambient laboratory temperature in paper bags, until their utilization (April 2012).

B. Methods

Only healthy seeds are selected for germination tests. It's were disinfected by sodium hypochlorite (1%) for 8 minutes, and then rinsed with distilled water to remove all traces of chlorine. Different concentrations of NaCl were prepared: 0.5 g / l, 1g / l, 1.5 g / l, 2g / l, 3 g / l, 4 g / l, 8 g / l, 16 g / l. For each concentration of NaCl, germination tests were carried out at different temperatures (5°C, 20°C, 25°C, 30°C, 35°C, 40 °C) in the dark in an oven Memmert Type. In parallel for each experiment, a control test was conducted.
For each test, 100 seeds of *Medicago arborea* divided into five lots of 20 seeds were used. Each lot of 20 seeds is placed in a sterile Petri dish containing two layers of filter paper moistened:
- by distilled water only for control test,
- by distilled water with different concentrations of NaCl for experiments.

For temperatures 5°C, 20°C and 25°C seeds are moistened with 2ml per day.