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Full Length Research Paper

Eucalyptus camaldulensis Dehnh. Offers Excellent Potential to Reduce NO_3^- Concentration in Groundwater

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Geologic and fertilizer N negatively impact the groundwater in the Gareh Bygone Plain (GBP) in southeastern I.R. Iran. Moreover, the floodwater used since 1983 for the artificial recharge of groundwater (ARG) to alleviate the water crisis in the GBP contains 60.30 ± 21.60 mg L^{-1} geologic NO_3^- on average. This has the potential to intensify the contamination. As the Agha Jari Formation, which supplies the NO_3^-contaminated runoff, covers 27680 km^2 in the southern I.R. Iran, an untold number of people will be affected with nitrate poisoning where such waters are inevitably used for the ARG. Dissolved NO_3^- concentrations were monitored monthly for a year in 30 shallow wells in a sandy-gravelly aquifer in the GBP to quantify the effects of different landuse and *Eucalyptus camaldulensis* Dehnh. on nitrogen retention. A laboratory experiment was also conducted to verify the potential nitrate absorption of the eucalyptus and NO_3^- surface adsorption potential of the calcareous sand. Our results suggest that landuse and management practices significantly impacted groundwater NO_3^- (*P<0.05*), EC (*P<0.01*), and its pH (*P<0.05*) in the GBP. Floodwater NO_3^- was highly reduced when passed through the vadose zone where NO_3^- was absorbed by the eucalyptus roots and adsorbed by the free $CaCO_3$ in the calcareous alluvium. The amount of NO_3^- retained by the roots was 770 fold the amount retained by the free $CaCO_3$ particles on the surface area basis. Therefore, establishing forested filter zones within the ARG systems using more efficient and native plant species supplies safer drinking water for rural inhabitants and desert-dwellers.

Keywords: *Eucalyptus camaldulensis* Dehnh, NO_3^- Concentration in Groundwater**.**

Abbreviations

AJF, Agha Jari Formation; ARG, Artificial recharge of groundwater; BZB, Bisheh Zard Basin; BZR, Bisheh Zard River; DO, Dissolved oxygen; GBP, Gareh Bygone Plain; MCL, maximum contaminant level; PV, Pore volume; SSA, Specific surface area.

INTRODUCTION

Elevated NO_3^- levels in groundwater is a major environmental concern. A survey by Mueller et al. (1995) found 1% of community wells and 9% of rural domestic wells had NO_3^- concentrations above the 45 mg L^{-1} maximum contaminant level (MCL) that the USEPA has established as the safety limit for drinking water (US

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Environmental Protection Agency, 2000; Sparks, 2003). The fraction of contaminated wells was as high as 26% in areas with land use under intensive agriculture. However, Walton (1951) reported that USEPA had set 45 ppm NO_3^- as the limit for safety, based on the dose that can produce methemoglobinemia in infants. To the best of our knowledge, the Iranian Environmental Protection Organization has not set a standard for N in groundwater. Our study may prove to be landmark in this subject matter. What is important is for the people to realize that not all nitrates in groundwater is from applying the N-fertilizers to crops. The reduction of nitrogen deposition remains a crucial factor in avoiding increased nitrate leaching into groundwater resources.

Nitrate is highly leachable and readily moves with water through the soil profile. If there is excessive rainfall or over-irrigation, nitrate will be leached below the rooting zone and may eventually reach groundwater. High NO_3^- concentrations in drinking water supplies can cause methemoglobinemia, the blue baby syndrome, a condition found especially in infants under six months, and have been cited as a risk factor in developing gastric and intestinal cancers in adults (Ritchie, 1990; Sakadevan et al., 2000; Flite III et al., 2001; Kumar et al., 2004). The presence of a strong relationship between NO_3^- concentration levels in drinking water and human health issues has been established (L'Hirondel and L'Hirondel, 2001).

Surface and subsurface flows are known as the main NO_3^- pathways to contaminate groundwater in both the permanently and intermittently saturated soils (Sharma, 1997). In most cases, recharge runoff emanating from the soil surface, and leaching followed by infiltration, are the main pathways for pollutant transport to groundwater (Ma and Spalding, 1997). Vertical movement of pollutants in recharge areas may occur through preferential flow paths and cause their rapid mixing in the aquifer (Heiman et al., 1997; Stamn et al., 1998). As NO_3^- is highly mobile and very soluble, the recharge events, either by rainfall or irrigation may create infiltration pulses of downward flowing water and NO_3^- (Bauld, 1996). Nitrate transport through the soil profile is highly variable and is influenced by many factors including vegetation, landscape, soil type, and tillage system (Gasser et al., 2002; Rowe, et al., 2006).

Solute dilution before recharging (Altman and Parizek, 1995; Ma and Spalding, 1997) depends on the type of flow through the unsaturated soil profile, which also depends on the rainfall intensity, soil water content, infiltration rate and the depth to water table (McDougall and Pyrah, 1998). As a result, NO_3^- concentrations will decrease in the aquifer where the source of contamination is removed, or substantial amounts of freshwater are injected into the aquifer through natural or artificial recharge processes. Downward movement of water beyond the rooting zone results in displacement of certain amounts of NO_3^- in the soil profile (Singh and Sekhon, 1978; Schuh et al., 1997; Harter et al., 1998), especially in the arid and semi-arid regions (Stokstad, 2003; Walvoored et al., 2003). In fact, infiltrating water displaces antecedent moisture, and the recharge to the aquifer maybe the water from older infiltration events (Horton and Hawkins, 1965). Dissolved ions may also be displaced rapidly in the soil profile due to the presence of the relatively immobile soil water content, which occurs in pore spaces that are not well connected to the main system, but reduce the transient time through the vadose zone, so that recharge occurs more rapidly (Kruppa and Elrick, 1968).

As Iran is the land of flash floods, droughts, and *qanats* (underground galleries that collect and deliver groundwater to agricultural communities) (Kowsar, 1991), the artificial recharge of groundwater (ARG) is the most appropriate technology to supply freshwater, to control saline water intrusion into freshwater aquifers (Todd, 1980), to prevent land subsidence caused by lowering of piezometric surface (Todd, 1980), to reclaim the drastically disturbed lands (Kowsar, 2005), and to mitigate flooding-induced disasters (Kowsar, 1991), thus to sustainably manage the marginal drylands during recurrent and prolonged droughts (Mesbah and Kowsar, 2010). Realizing that the Land of Iran contains in excess of 5,000 km^3 of underground space, 11 fold the total volume of its mean annul precipitation (Kowsar, 2005), makes the ARG particularly attractive among water resources management alternatives.

We were not able to detect any N-containing mineral in the XRD patterns. However, our depth sampling through sandstone showed the highest NO_3^- concentration at a depth of 60 cm. As the Agha Jari Formation has been exposed to the atmosphere during the past 2 million years, we expeculate that the N-contained in the meteoric waters leached through the sandstone and accumulated at that depth. Weathering and capillary rise bring NO_3^- to the exposed surface, ready to be washed by the floodwater. The presence of geologic nitrogen ((Hill 1996), in the runoff of the Agha Jari Formation (AJF) (Yazdian and Kowsar, 2003) and a few other Miocene era deposits (Kowsar, unpublished data, 1986) that supply floodwater to a large number of ARG systems is both a boon to and the bane of the water users.

Nitrogen deficiency ranks right behind water shortage as the second-most constraint to crop production in the coarse–loamy sand of the plains, where their alluvium have been originated from the erosion of 27680 km^2 of the AJF in the Zagros Mountain Ranges in southern Iran(Yazdian and Kowsar, 2003); therefore, the floodwater is "N-enriched" in such terrains. On the other hand, the nitrate contaminated floodwater and agricultural runoff and leachate pose serious problems if indiscriminately used for human consumption. Therefore, great caution has to be exercised in utilizing such resources for the ARG.

A major deterrent to the success of any ARG project is the high turbidity of the floodwaters used in its implementation. Clogging of the vadose zone by the minute clay-sized particles is inevitable in such systems (Mohammadnia and Kowsar, 2003). As active and decaying roots interact with soil matrix under trees and improve water flux through preferential flow paths (e.g. root channels), therefore, planting of deep-rooted trees in the ARG systems is strongly advised. Biopores, particularly open-ended sowbug (*Hemilepistus shirazi* Schuttz.) burrows, improve infiltration and saturated hydraulic conductivity of the surface crust in these systems (Rahbar and Kowsar, 1997).

The ARG through floodwater spreading, a simple and economic technology, has provided freshwater for drinking and irrigation in the Gareh Bygone Plain (GBP), southeast Iran, since 1983 (Kowsar, 1991). It is informative to note that the dilution of the brackish and saline water by the ARG activities decreased the EC of the domestic and agricultural well water in the GBP from 0.29 to 3.25 fold decreased during the 1983-1996 period (Pooladian and Kowsar, 2000).

This study was conducted as an offshoot of the Aquifer Management Project (Kowsar, 2005), a nationwide initiative in the Islamic Republic of Iran. Provision of adequate safe water through recharging the overused and abused aquifers in the wet years, and optimizing the water consumption for sustainable agricultural production are the main goals of this project.

Groundwater in the GBP is contaminated with geologic and fertilizer N. As the mean NO_3^- concentration of the floodwater used for the ARG in the GBP is 60.30 ± 21.60 mg L^{-1} (Yazdian and Kowsar, 2003), therefore, it has the potential to intensify the N contamination of the aquifer; thus, the recharge water has to be filtered before reaching the aquifer to meet the USEPA's drinking water standard set for NO_3^-. Therefore, any cultural practice to reduce NO_3^- concentrations in drinking water could be advantageous. Peterjohn and Correll (1984) have reported 89% retention of N by riparian forests. In certain places of the GBP planted with *Eucalyptus camaldulensis* Dehnh. and irrigated with floodwater, groundwater contains the lowest levels of NO_3^- (6.12 mg L^{-1}), indicating the probable depletion of NO_3^- by N utilization.

As the level of NO_3^- in groundwater in each area is influenced by many previously mentioned factors, we hypothesized that afforestation in this sandy desert, where N is the primary limiting nutrient essential to biomass production, was the most important reason for nitrate reduction in the ARG systems. Highly significant correlations between forest productivity and N mineralization have been reported by Reich et al. (1997). The fast growing plant species that produce great biomass have the potential for ameliorating the environmental impact of excess NO_3^-, and could be used as a tool to mitigate groundwater contamination while benefiting farmers and other inhabitants in the region socially, economically and environmentally. The high productivity of *Eucalyptus camaldulensis* Dehnh. , which is physiologically active during the rainy season, and has manifested excellent adaptation to the region, makes it a good candidate for phytoremediation; however, its potential on NO_3^- removal from the soil is not well documented in the literature. Therefore, monitoring of groundwater under eucalyptus plantations is warranted to quantify the magnitude of impacts.

As $CaCO_3$ is the predominant mineral in the soil compartment of our recharge systems, and the adsorption of NO_3^- by $CaCO_3$ has been attributed to the steric compatibility of NO_3^- with the calcite crystal lattice, therefore, the relationship of NO_3^- concentration with the carbonate surface area could be important in defining the transport of that anion in the soil (Jurinak and Griffin, 1972).

According to Singh and Sekhon (1978), the magnitude of NO_3^- adsorption is not proportional to the amount of $CaCO_3$ in a calcareous soil. Possibly, the difference in the specific surface area (SSA) of $CaCO_3$ in different soils governs their NO_3^- adsorption potential. Although granular $CaCO_3$ predominates in the soil of different landuse in the GBP (Table 1), it is speculated that the fine powder form is predominant in the sedimentation basins of the recharge systems due to the deposition of the suspended load. The smaller the size, the larger is the SSA of $CaCO_3$ particles. Quantification of this remediation potential helps a more precision design of the ARG systems in the Ca-rich alluvial plains.

According to the United Nations University's study on groundwater vulnerability as a part of an international Programme called Groundwater and Human Security – Case Studies project (GWAHS-CS) carried out in the Shibkouh County of Iran comprising GBP (2008-2010), the last decade groundwater data were documented (www.ehs.unu.edu). Results obviously confirmed impacts of different landuses on the groundwater quality and quantity between years (data not shown). The measured rainfall during the study period was 260.5 mm; however 6,827,000 m^3 floodwater were diverted into the FWSS to recharge the aquifer.

Flash floods are the only means that supply water to our recharge systems. Furthermore, we have been artificially recharging the groundwater since Jan. 1983. Therefore, we were concerned with only one source of water during the experiment. However, in the earlier days, the recharge water diluted the brackish groundwater significantly.

Since floodwater is the sole source for the ARG in the GBP, and NO_3^- concentration in the groundwater shows high spatial and temporal variations in that area, this study was devised to reveal the underlying mechanisms affecting groundwater NO_3^- concentration**.** The aims of this study were thus (i) to identify the effects of different landuse and management practices on nitrate concentrations in groundwater and (ii) to evaluate the NO_3^- removal potential

Table 1. Analyses of 21 surface soil samples collected from different landuse systems in the study area. SB denotes sedimentation basin and S denotes strip of sedimentation basin.

Sampling location	Clay, %	Silt, %	Sand ,%	C.C.E.,%	Gypsum,%	pH	EC, dS/m	SO_4^{-2} meq/L	OM %	OC %	N %	CEC Cmol(+)kg^{-1}	AEC Cmol (-)kg^{-1}	Ads.$_{max}$ (mg L^{-1} NO_3^-)
Zone 1	8.40	14.60	77.00	42.03	Trace	8.05	0.43	1.12	0.32	0.18	0.01	6.00	0.50	17.20
Zone 1	7.40	6.60	86.00	42.44	Trace	8.01	0.46	1.75	0.48	0.27	0.02	4.95	0.25	15.30
Zone 1	10.40	8.00	81.60	42.12	Trace	8.17	0.35	7.41	0.35	0.20	0.01	5.30	0.30	13.47
Zone 1	7.40	12.00	80.60	39.65	Trace	8.16	1.24	0.79	0.32	0.18	0.01	4.00	0.20	14.83
Zone 1	10.40	10.00	79.60	40.18	0.05	7.97	4.53	37.99	0.54	0.31	0.02	6.70	0.30	13.47
Zone 2	8.40	8.60	83.00	38.46	Trace	8.24	1.80	10.66	0.47	0.27	0.02	4.20	0.20	15.45
Zone 2	7.40	7.60	85.00	39.05	Trace	7.92	0.51	1.17	0.44	0.25	0.02	4.95	0.20	12.87
Zone 2	11.40	7.60	81.00	37.28	Trace	8.02	3.20	Trace	0.38	0.22	0.19	4.69	0.20	13.79
Zone 2	9.40	7.60	83.00	38.49	Trace	8.06	1.66	9.89	0.35	0.20	0.17	6.00	0.40	16.97
Zone 2	9.40	8.60	82.00	39.65	Trace	8.13	0.37	22.07	0.35	0.20	0.01	5.00	0.25	15.26
Zone 3 (SB_1-S_1)	30.40	28.00	41.60	31.63	Trace	7.98	0.39	1.29	0.79	0.46	0.04	10.57	0.75	22.72
Zone 3 (SB_1-S_2)	22.40	12.60	65.00	32.00	Trace	7.76	8.66	2.66	1.10	0.64	0.05	9.90	0.65	20.32
Zone 3 (SB_1-S_3)	7.40	9.60	83.00	38.93	Trace	7.98	0.56	1.64	0.44	0.25	0.02	5.60	0.20	14.99
Zone 3 (SB_1-S_4)	13.40	9.00	77.60	35.29	Trace	8.05	0.39	0.97	0.48	0.27	0.02	6.00	0.30	17.51
Zone 3 (SB_1-S_5)	8.40	8.00	83.60	39.02	Trace	8.00	0.52	1.74	0.35	0.20	0.01	6.20	0.20	15.45
Zone 3 (SB_1-S_6)	9.40	8.60	82.00	32.38	Trace	7.84	0.80	5.77	0.57	0.33	0.02	7.50	0.30	18.20
Zone 4	13.40	27.60	59.00	41.84	0.17	7.81	8.41	Trace	0.60	0.35	0.03	6.85	0.20	15.20
Zone 4	4.70	17.80	77.50	40.95	Trace	8.06	0.64	2.20	0.60	0.35	0.03	2.61	0.20	13.22
Zone 4	4.40	16.00	79.60	40.86	Trace	7.97	3.11	25.81	0.38	0.22	0.02	3.40	0.20	12.00
Zone 4	6.40	10.00	83.60	39.34	Trace	7.86	3.10	24.15	0.35	0.20	0.01	4.30	0.25	11.90
Zone 4	11.40	25.00	63.60	42.12	0.31	7.69	11.63	68.70	0.79	0.46	0.04	6.90	0.30	13.62

of *Eucalyptus camaldulensis* Dehnh. and calcareous sand from the recharge water at bench scale and *in situ*.

MATERIALS AND METHODS

Site Description

This study was conducted at the 2030 ha Kowsar Floodwater Spreading & Aquifer Management Research, Training and Extension Station (28° 38' N, 53° 55' E, 1140 m above mean sea level) in the GBP, 200 km southeast of Shiraz, I.R. Iran on a debris cone and an alluvial fan formed by the ephemeral Bisheh Zard River (BZR) that drains the 192 km^2 Bisheh Zard Basin (BZB, Figure.1). The area is characterized by a continental Mediterranean climate with a very low and highly variable rainfall with a mean annual precipitation of 243.3 mm that occurs mostly in fall and winter, and the Class A pan evaporation of 3200 mm (Kowsar and Pakparvar, 2004). The BZB is a

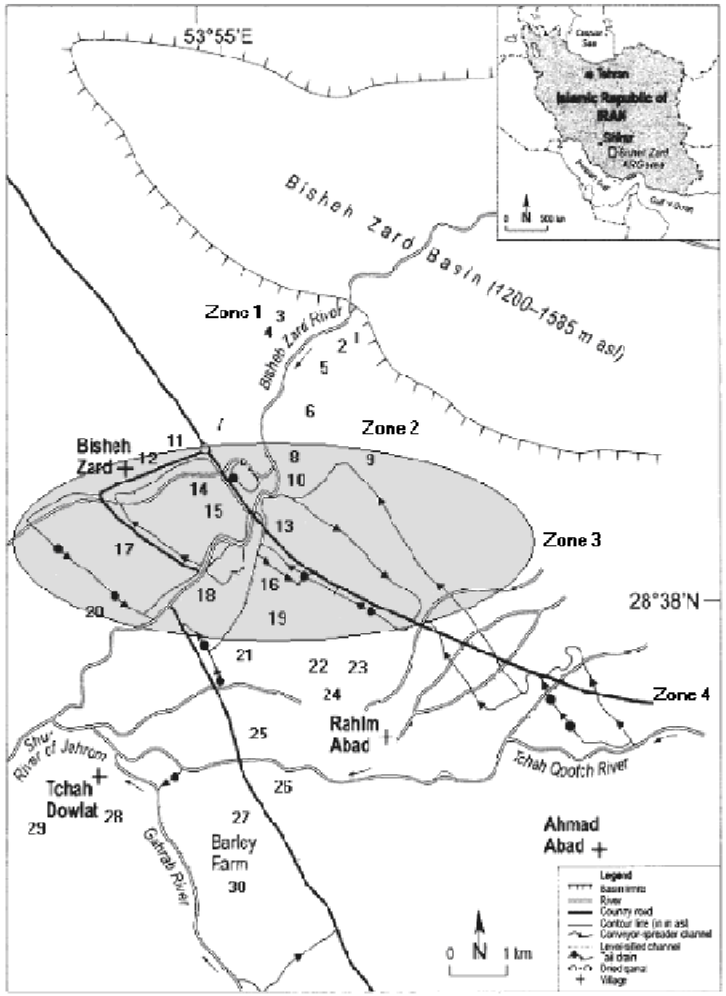

Figure1. Sketch map of the Gareh Bygone Plain (GBP) artificial recharge of groundwater (ARG) systems. Numbers denote production wells; the grey oval denotes ARG systems in the study area.

northwest to southeast syncline formed by the tectonic movement of the Zagros Mountain Ranges during the Mio-Pliocene time in the AJF. This formation consists of rhythmically interbedded calcareous sandstones, and slightly weathered gypsum–veined marls and grey to green siltstones (James and Wynd, 1965).

In arid and semiarid areas such as the study site, the inorganic carbonate accumulated in soils by pedogenic formation or inherited from calcareous parent material may contain as much as 40% or more of calcite equivalent (Nelson and Sommers, 1996). Variability in alluvial plains' sediment and soil specification is a rule rather than exception, as the floodwater may originate in different sub-basins containing different outcrops of the same formation (Table 1). Gypsum veins are found in the Agha Jari Formation, but folding has caused different percentage of land to expose gypsum seams. This accounts for other soil characteristics as well. Since the original soil of the GBP is very low in organic matter, therefore, most of the CEC and AEC should be attributed to the clay minerals. It follows from the above paragraph that the variability could be attributed to the flooding event history a few thousand years ago (may be millions of years).

Amounts of soil calcium carbonate for 21 samples collected from different land uses represented in Table 1 indicate the presence of a calcareous aquifer underneath the ARG systems. The original soil of the study site is classified as a coarse-loamy skeletal carbonatic (hyper) thermic, Typic Haplocalcids (Soil Survey Staff, 1999).

The BZB terminates at the apex of a debris cone that extends to an alluvial fan east of the GBP (Figure.1). The known thickness of the alluvium in the GBP ranges from practically none on the eastern margin of the debris cone to 43 m midway the width of the plain, 4 km downstream from the gorge that forms the outlet of the BZB (Pooladian and Kowsar, 2000; Kowsar and Pakparvar, 2004). Due to the construction of the ARG systems, a considerable portion of the flow of the BZR is diverted into the systems and the surcharge leaves the study area for environmental purposes without passing through the farm fields.

The study area was divided into four distinct zones based on their landuse systems: Zone 1 begins from the debris cone apex adjacent to the BZB outlet and extends to the farming areas (non-anthropogenic activity area, as the control); Zone 2 is located downstream Zone 1 on the alluvial fan and is under agricultural activities; Zone 3 is mostly occupied by the ARG systems and includes the afforested basins planted to *Eucalyptus camaldulensis* Dehnh. and non-afforested basins and; Zone 4, located at the lower end of the alluvial fan, is under intensive farming activities (Figure.1). The average depths to the watertable during the study were 12, 25, 23 and 26 m for zones 1-4, respectively. Eleven flood events occurred during the study period. For further information, please refer to Mohammadnia (2010).

Groundwater Monitoring and Laboratory Treatment

Thirty shallow production wells, located in different landuse systems, were selected based on the groundwater flow direction in the GBP (Figure1). Three 500 ml water samples were taken monthly from the pump head of each well over a 12 month period and analyzed for dissolved oxygen (DO), pH, EC and NO_3^- using portable equipment. Concentration of NO_3^- was measured by the cadmium reduction method (Greenberg et al., 1992) using a HATCH-DR2400 portable UV-visible spectrophotometer at a wavelength of 543 nm. Dissolved oxygen was measured by the electrometric method in the field using a portable oxygen meter (Model WTW 3, OXI 320/SET). Groundwater EC and pH were measured in the field using an EC meter (Model WTW LF, 340) and a pH meter (Model WTW pH, 340). Differences between NO_3^- concentration, EC and pH as the main groundwater characteristics were evaluated between zones using the Student's *t* test at the probability levels of $P<0.01$ and $P<0.05$ (SAS Institute, 1999).

Measurement of nitrate vertical distribution in the vadose zone of the control and ARG sites.

To compare NO_3^- vertical distribution of the vadose zone in the ARG system (BZ_4) and non flooded areas as control, a systematic soil sampling through two 100 cm diameter wells was made from soil surface to a depth of 23 m. Wells were located on the 1140 M contour to provide same sedimentation sequences of the vadose zone. Three soil samples were collected at each 1 M intervals and mixed together to obtain a composite sample. Nitrate concentration of each sample was measured using micro diffusion method based on Khan et al. (2000) and Mulvaney (1996).

Soil and Plant Leaf sampling and Laboratory Treatment

Soil samples were taken from the 0-30 cm depth of different landuses of the study areas. For zones 1, 2 and 4, samples were taken from corners and cross points of diameters of a rectangle covered main part of each zone. For zone 3 which is occupied by FWSS, samples were taken from 6 continuous sedimentation basins so called strips and act as recharge basins. Samples were kept in plastic labeled bags in a cool dry place for less than 5 hours to transfer to central analytical lab located in Shiraz and then were air dried and passed through a 2 mm sieve. Soil texture was determined by the pipette method (Gee and Bauder, 1986) Total nitrogen content of the soil was measured by the regular Kjeldahl method (Bremner, 1996). The soil inorganic nitrogen forms were measured using the micro diffusion method in which soil extracts obtained with 2 *M* KCl using a soil/solution ratio of 1:10, transferred to a Mason jar and were treated with proper amounts of MgO and Devarda's alloy based on Khan et al. (2000) and Mulvaney (1996). Soil organic carbon and calcium carbonate content were measured by the rapid dichromate oxidation technique (Nelson and Sommers, 1996) and acid-neutralization method (Bundy and Bremner, 1972), respectively. Soil pH was measured using a 1: 5 proportion of air-dry soil and deionized(DI) water to prepare a suspension and determine its pH electrometrically based on Thomas (1996), and EC of the soil extract and groundwater samples was determined by rinsing the cell of the conductance meter with one or more portions of sample, based on Rhoades (1996). The gypsum concentration of the soil was measured by the quick method followed by washing and stirring 10 g of the soil sample with 100 mL of warm water and collecting dilute water extract 1: 5 based on Leoppert and Suarez (1996).

Soil and sediment CEC was measured using sodium acetate trihydrate ($NaOAc.3H_2O$; Rhoades, 1982). Soil and sediment samples for CEC were taken from zone 1 and 1st strip of zone 3, respectively. The anion exchange capacity (AEC) of the soil sample was measured using the Rhoades method (1982).

Leaf samples of *Eucalyptus camaldulensis* Dehnh., which was planted in the flooded sedimentation basin (BZ_4), and non-flooded area of BZ_4 (Figure. 1),were randomly collected from 10 trees in each treatment. Fresh leaves were dried at about 60˚C (Beaton Jones and Steyn, 1973) to a constant weight. Leaves were ground to a fine powder and analyzed for nitrogen content using the micro-Kjeldahl method (Keeney and Nelson, 1982).

Nitrate Uptake Experiment

To study NO_3^- uptake by *Eucalyptus camaldulensis* Dehnh. in a saturated condition, simulating what occurs in the afforested ARG systems during the recharge activities, a leaching experiment with saturated packed soil columns was conducted using planted and non-planted treatments in three replicates. Average NO_3^- concentrations for collected leachate fractions of treatments (totally 30 leachate fractions) were used to obtain NO_3^- breakthrough curves. The soil used in this experiment was collected from the forested area planted with *Eucalyptus camaldulensis* Dehnh. As soil of the sampled area was structureless, its measured bulk density inside the columns was approximately the same as that of the soil in the field. The PVC columns were 30 cm long with an inside diameter of 10 cm. They were filled up with < 2 mm of sieved soil to a depth of 20 cm. Two, 3 cm washed medium sandy layers were laid both on the top and at the bottom of each soil column and two pieces of filter paper (Wattman, 42nm) were also used at the bottom of each column. The volumetric water content of the columns was determined from the difference in weight before and after saturation. Pore water velocity was calculated as column solution divided by volumetric water content (Qafoku and Sumner, 2001). This also constituted the soil pore volume (PV). To remove the deleterious effects of soil living microorganisms on nitrogen content of the columns, a chloroform solution of 1000 ml was leached through each packed soil column. Soil saturation was done by filling the columns with water from the bottom. One, 1-year old eucalyptus seedling was planted in each of the three columns (treated), and the remaining three were left as the control. Columns were watered with a water sprayer to inhibit soil surface disturbance at a 3-day interval with NO_3^- free water for one month to ensure the establishment of the seedlings. The columns were installed on ceramic funnels to facilitate leachate collection. Soils in the columns were washed with deionized water until there was no NO_3^- in the leachate. To run the leaching experiment, the columns were saturated with deionized water from the bottom, after which a flush of totally dissolved of 50 mg NO_3^-, as Ca $(NO_3)_2$, was added in a 10 ml aliquots across the top surface of each column. The NO_3^- was eluted by continued addition of deionized water using a 5 cm constant hydraulic head. Leachate fractions were collected at 0.5 pore volume intervals (1 PV

= 105 mL) and analyzed for NO_3^-concentration using the cadmium reduction method (Greenberg et al., 1992). Leachate sampling was terminated after passing 5 PV of water through the columns and leachate fractions were collected for both planted and non-planted columns. This experiment lasted 18 hours on average. Nitrate concentrations of 60 collected 0.5 PVs obtained from both planted and non-planted columns, were statistically compared using the paired t-test. To calculate the NO_3^- retardation factor (R), a batch adsorption isotherm for soil used in the columns was carried out in three replications using 5, 10, 20, 30, 40 and 50 mg/L NO_3-N solutions and the batch K_d (adsorption coefficient) was calculated.

The root surface area of the eucalypt seedlings was estimated using the Smika and Klute (1982) method. The planted soil columns were first soaked in distilled water for one hour and the root-attached soil material was removed while the integrity of the roots was preserved. A digital image of the rooting system was scanned to provide a black and white two dimensional picture, which was processed using the ILWIS ver. 3.4 (http://www.downloadatoz.com/multimedia-design_directory/ilwis/) to count number of black (root hairs) and white (soil environment) pixels. As the average diameter of the root hairs was 0.1 mm, the black pixels were oriented to form rectangular figures 0.1 mm wide and having the length of the root hairs. The area of all rectangles (A), times π approximates the root surface area.

Nitrate Adsorption Experiment

Batch adsorption isotherm was carried out to find the NO_3^- adsorption maximum (Ads_{max}) for 21 surface soil samples collected from different landuse systems, using 0, 5, 10, 20, 30, 40, 50 mg L^{-1} NO_3^- solution from source KNO_3. Characteristics of the samples are shown in Table 1. The samples were first placed into a column and washed with deionized water to ensure there was no NO_3^- in the leachate. Five g air-dried, <2 mm sieved washed samples was weighed into centrifuge tubes. Nitrate solutions were added and the volume in each centrifuge tube was made to 40 ml. Tubes were shaken for 2 hours and kept overnight in an isolated room at 25 ˚C. Tubes were then shaken again for 30 minutes, centrifuged at 2000 rpm, and the NO_3^- concentrations of supernatants were measured by the cadmium reduction method (Greenberg et al., 1992). Least square linear regression analysis was used to describe relationships between 21 soils' AEC vs. Ads_{max} for NO_3^-. The SSA of the mentioned soil samples were calculated using the following equation (Holford and Mattingly, 1975):

$$SSA = 5.8\left(\frac{100}{\%CACO_3} - 1\right)$$

(Biggar and Nielsen 1976).

RESULTS

Measurements of Groundwater NO_3^-, EC, pH and DO

Effect of different landuses on NO_3^-, EC, and pH of the groundwater extracted from the 30 production wells for 12 months is presented in Table 2. Vertical distribution of NO_3^- in the vadose zone of the control and the ARG sites are depicted in Figure.2. Oxidizing conditions was predominant

Table 2. Effect of different landuses on NO_3^-, EC, and pH of the groundwater extracted from 30 production wells (mean ±1SD). Each well was sampled for 12 months.

Items \ Zones	Z1	Z2	Z3	Z4	Sig.
NO_3^- (mg L^{-1})	18.31 ± 11.27^{bc}	38.08 ± 21.67 ^{a}	7.10 ± 3.99 ^{c}	31.34 ± 27.87 ^{ab}	**
EC (dS m^{-1})	2.91 ± 0.22^{a}	3.88 ± 0.22 ^{a}	1.64 ± 0.28 ^{b}	4.03 ± 2.48 ^{a}	**
pH	7.49 ± 0.27 ^{a}	7.29 ± 0.94 ^{b}	7.47 ± 0.27 ^{a}	7.44 ± 0.14 ^{a}	*

* = P<0.05 ** = P<0.01

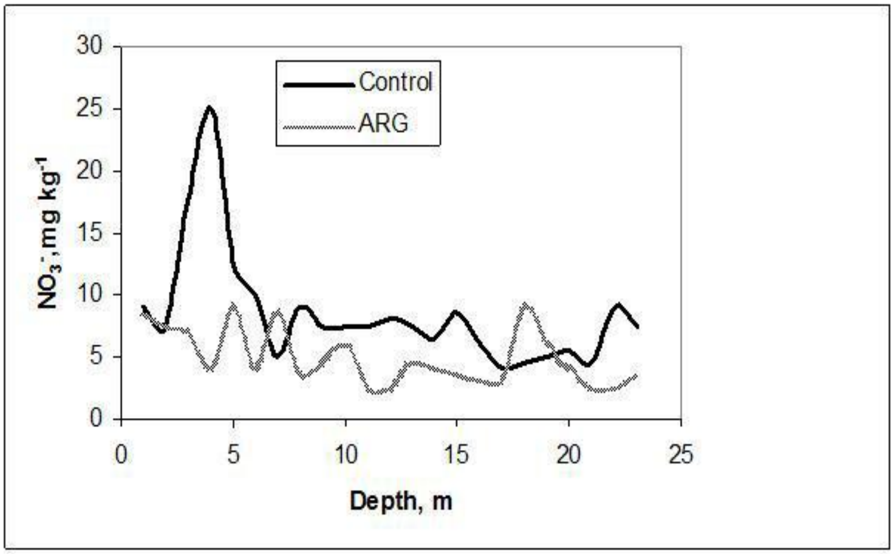

Figure 2. Nitrate vertical distribution in the soil profile of the control and ARG sites.

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throughout the aquifer, which in turn provided a suitable condition for NO_3^- stability; therefore, the possibility of reduction processes was negligible. Average NO_3^- concentration in Zone 1 was 18.31 mg L^{-1}, which is lower than the permitted level in drinking water by the USEPA. A dramatic increase of 91.18% (*P<1%*) in the average of NO_3^- concentration was measured for Zone 2, as compared with Zone 1 (Table 2). A sharp decrease in the groundwater NO_3^- was observed as it transitioned from Zone 2 (38.37 mg L^{-1}) to Zone 3 (7.10 mg L^{-1}). The lowest NO_3^- concentration was discovered in the aquifer located under the afforested basins in Zone 3 (6.12 mg L^{-1}). The average NO_3^- concentration in Zone 4 (31.33 mg L^{-1}), was 4.41 times higher than that of the upstream zone (*P<1%*). High DO (4.08 mg L^{-1}), provided NO_3^- stability condition even in Zone 4 located at the lower end of the GBP. The maximum NO_3^- concentration in the aquifer was registered for well 30 in Zone 4 (94.45 mg L^{-1}).

Measurements of Roots and $CaCO_3$ Surface Area

The root surface area of the column-planted eucalypt seedlings used in this experiment was 3620 cm^2, and that of the $CaCO_3$ particles in the same column (number 11, Table 1), containing the least amount of $CaCO_3$ and the largest $Ads_{.max}$ for NO_3^- adsorption was 11.2268×10^6 cm^2.

Measurements of NO_3^- Absorption by Roots and Adsorption by $CaCO_3$

Although nitrate concentration in the leachate fractions of the non-planted saturated columns was not significantly greater than that of the planted ones (Table 3), almost all of the NO_3^- leached at the first PV of the soil for the non-planted column. This occurred for the planted columns at more than the first PV (1.5 PV on average), suggesting

Table 3. Paired t-test for NO_3^- concentration of each 0.5 PV fractions for planted and non-planted columns.

		Paired differences					t	df	Sig. (2-tailed)
		Mean	Std. deviation	Std. mean error	95% Confidence interval of the difference				
					Lower	Upper			
Pair 1	y1-y2	-2.00733	22.74202	4.15211	-10.4993	6.48468	-0.483	29	0.632

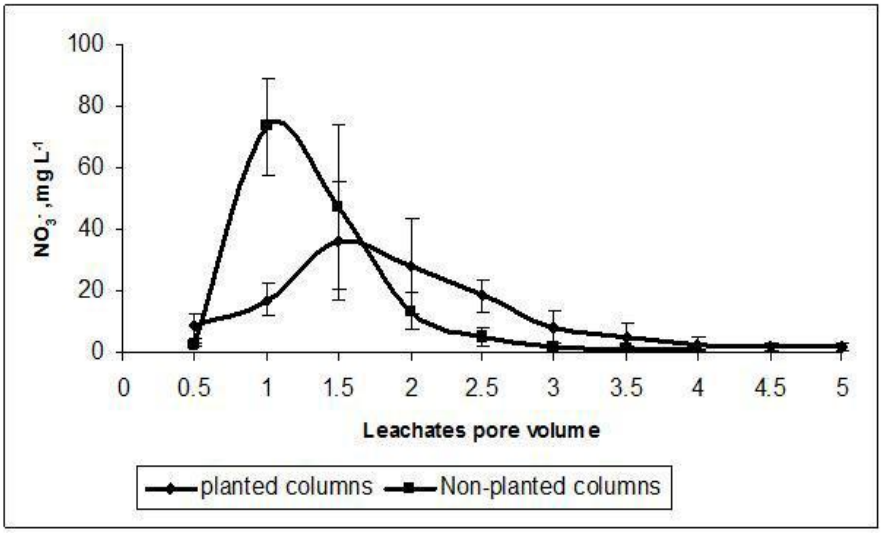

Figure 3. Breakthrough curves of comparative average of NO_3^- concentration in the leachate fractions of saturated leaching columns. Bars denote ±1 SD of the mean.

greater NO_3^- retention and utilization by plants, thus its reduction by leaching (Figure. 3).

It is important to note that the NO_3^- input to each column was 221 mg; the average output of the planted columns was 128 mg, and that of the non-planted columns output was 148 mg. Therefore, 148-128=20 mg was absorbed by the eucalyptus seedling. Moreover, 221-148=73 mg was adsorbed by the calcareous particles in the columns; this implies that 73 mg of NO_3^- in the planted column was adsorbed on 11.2286×10^6 cm^2 surface area of $CaCO_3$ (density, $\rho_1 = 6.50\times10^{-6}$ mg NO_3^-/ cm^2). On the other hand, 20 mg of NO_3^-, which represent the net uptake rates (the difference between influx and efflux transport (MacKowen et al., 2009), was absorbed by 3620 cm^2 of the root surface area of the seedling (density, $\rho_2 = 0.005$ mg NO_3^-/ cm^2). The ratio of ρ_2/ρ_1 indicates that the eucalyptus rooting system had absorbed 770 times more NO_3^- than adsorption by the $CaCO_3$ particles on the surface area basis during the experiment that lasted 12 hours on average.

In the non-planted elution curve obtained for the average of leached NO_3^- concentration in each PV, the breakthrough of NO_3^- occurred at one pore volume (Figure. 3). The area under each peak represents the dissolved NO_3^- through the columns as infiltration continues. The lack of symmetry of the curves in Figure.3 indicates NO_3^- retention in the soil columns. The long descending portion of each curve arises from releasing of NO_3^- in the sorption sites, with the length dependent on the amount of NO_3^- adsorbed (Singh and Sekhon, 1978). In fact, as the peak of the curves diminishes, the trailing portion of the elution curve becomes more pronounced because of the increased NO_3^- retention. As mentioned earlier, the area under the elution curves represents the amount of NO_3^- eluted from the columns. Thus, the lower average area under the curve of the planted columns as compared to the non-planted columns proves more NO_3^- retention in the planted columns due to plant uptake.

DISCUSSION

Predominant oxidizing conditions throughout the vadose zone and the aquifer eliminated the reducing environment for the nitrate. Using equations (Altman and Parizek 1995; Batu 2006), (Freeze and Cherry, 1979), and considering the minimum dissolved O_2 (2.25 mg L^{-1}) and a pH of 7.97 in the groundwater of Zone 1, the electron activity (pE) for the groundwater would be:

$pE = 20.78 + ¼ \log (PO_2) – pH$ (Altman and Parizek 1995).

in which the concentration of DO is converted to PO_2, using Henry's law as below:

$PO_2 = O_2$ dissolved / KO_2 (Batu 2006).

and taking KO_2 at 25 ˚C as 1.28 ×10^{-3} mol / bar, we obtained the pE of 13.62, which is still large enough to be placed in the upper limit for oxidized water conditions in the pE-pH diagrams (Freeze and Cherry, 1979). This indicates the oxidizing tendency of groundwater, even at the lowest DO concentration. As denitrification occurs at pE of about 4.20 (Freeze and Cherry, 1979), and the water would be devoid of dissolved O_2 at this redox potential, therefore, the possibility of denitrification in the aquifer was negligible, indeed.

Increasing NO_3^- concentration in the groundwater as it flows from Zone 1 (18.31 mg L^{-1}) to Zone 2 (38.37 mg L^{-1}) (*P<1%*) is quite predictable due to the application of N-fertilizes in the farm fields followed by leaching due to irrigation. The same conclusion is logical as groundwater passes through Zone 3 (7.10 mg L^{-1}) to Zone 4, (31.33 mg L^{-1}), which is highly affected by intensive agricultural activities. In Zone 3, which is mostly occupied by the ARG systems that receive a yearly mean of 10 million m^3 of floodwater containing 60.30 ± 21.60 mg L^{-1} NO_3^- (Yazdian and Kowsar, 2003), remediation by *Eucalyptus camaldulensis* Dehnh. decreases the NO_3^- concentration in that part of the aquifer to an average of 7.10 mg L^{-1}. This could be concluded from the data presented in Table 2. Average total N content of the *Eucalyptus camaldulensis* Dehnh. leaves from the flooded area (1.04%) was larger than that of the non-flooded area (0.84%).We did not perform any statistical analysis on this part of the study as we were not certain of the subsidization level of the nutritious sediment in the flooded basins to the total N content of the leaves. Even though Jolley et al. (2010) maintain that sedimentation in the highly disturbed plots of a riparian forest had lower N mineralization rates, as the suspended particles in our study contain inorganic NO_3^- (Yazdian and Kowsar, 2003), we believe that they influence the biogeochemical processes in the sedimentation basins. As sedimentation basins, partly planted to *Eucalyptus camaldulensis* Dehnh., form the predominant landuse of Zone 3, therefore, the most probable mechanism by which NO_3^- was removed from the vertical recharge flow was likely due to the uptake of NO_3^- in the rooting zone of these trees that penetrated the phreatic fringe, even the watertable, at a depth of 23^+ m, and the adsorption by calcareous soil particles.

The predominant effects of landuse on NO_3^- concentration, EC and pH were statistically analyzed using the Student's *t* test at the probability levels of *P<0.01* and *P<0.05* (SAS Institute, 1999) and are shown in Table 2, confirming the positive effects of the ARG activities and phytoremediation on groundwater decontamination.

In agreement with the findings by Mishra and Misra (1990), it is observed that the peak concentration of solutes in the leachate decreases and the tailing at the right-hand side of the breakthrough curve increases with an increase in the value of the retardation factor (R). The higher the value of R, the greater is the tendency of the soil to adsorb the ions and the longer is the tailing of the breakthrough curve. The initial time of the breakthrough curve is not influenced by the value of R. The retardation factor was computed using equation (Batu, 2006):

$R = 1 + \rho b K_d / \theta$

where K_d is the batch K_d and θ is the volumetric water content. The value of K_d can be estimated directly from the slope of the linear adsorption isotherm of the batch equilibrium study (Parker and van Genuchten, 1984). Taking ρb = 1.4 g.cm^{-3}, K_d = 0.34, and θ = 0.35 as the average amounts for planted saturated soil columns, gives R = 2.39, which is effective enough to retain NO_3^- (Różkowski et al., 2005); and decontaminate water in a densely rooted zone.

The convection diffusion coefficient, D (cm^2 h^{-1}), has been reported to be a function of solute velocity, V (Batu, 2006). Biggar and Nielsen (1976) have formulated D as:

$D = 0.6 + 2.93 V^{1.1}$ (Beaton et al., 1973).

Taking V equal to 2.90 and 2.20 cm h^{-1} for non-planted and planted columns, respectively, gives D = 10.05 and 7.57 cm^2 h^{-1} for the mentioned columns, respectively. As D is a function of the solute velocity, the breakthrough curves move to the left with an increase in the value of D, and an associated decrease in peak concentration (Mishra and Misra, 1990). In fact, the higher the velocity, the earlier the peak appears. Therefore, the peak concentration appeared earlier in the non-planted columns as compared with the planted ones.

As the clay-sized particle content of the 1st and 2nd basins of the ARG systems differed from that of others (30.40 and 22.40 %, respectively), we decided to find the effects of this difference, and also that of the calcium carbonate content of the soils on NO_3^- adsorption. Both NO_3^- and CO_3^{-2} anions have planar configurations with similar dimensions. In fact, NO_3^- as an indifferent anion should be electrostatically adsorbed and should not form inner-sphere complexes with surface reactive groups (Qafoku et al., 2000). According to McBride (1994), as the weak heat of adsorption is a characteristic of physical adsorption, in which the bonding interaction is not very energetic (<10 Kcal/mol of adsorbate), NO_3^- might be weakly adsorbed on the soil colloid surface.

Relatively significant relationships were obtained between $Ads._{max}$ of NO_3^- and AEC (r^2=0.68) (Figure. 4) in the adsorption isotherms. On the other hand, the nitrate adsorption on the surface of $CaCO_3$ (Jurinak and Griffin, 1972; Singh and Sekhon, 1978) indicates its relationship to the $CaCO_3$ SSA in certain conditions.

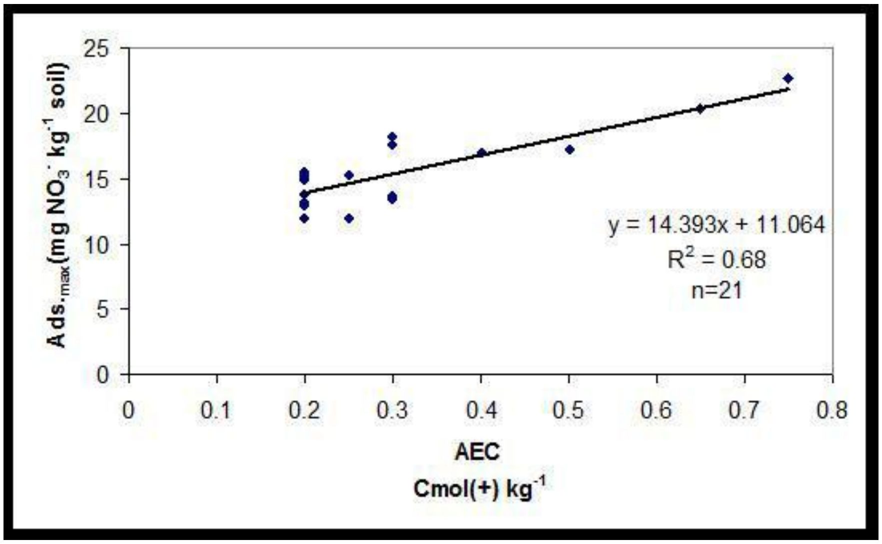

Figure 4. Average maximum adsorbed NO_3^- vs. each soil sample's AEC.

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Although soil samples, which were collected from 1st and 2nd strips of first sedimentation basin (SB_1-S_1 and SB_1-S_2) of the ARG system, contained lower amounts of $CaCO_3$ (31.63% and 32.00%, respectively) as compared with the other samples (Table 1), their SSA were larger (12.53 and 12.32 $m^2\ g^{-1}$, respectively). Thus, they represent a larger $Ads_{.max}$ for NO_3^- adsorption, 22.72 and 20.32, respectively. The very high root absorption rate of the eucalyptus seedlings as compared with that of the calcareous sand on the surface area basis indicates the excellent potential of this species as a NO_3^- remediator. The closeness of bench scale test results, using a growth medium devoid of chloroform sensitive microorganisms, with those of a natural system that provided the soil microorganisms with a unique source of N was remarkable. This revealed that both roots and calcareous sand, definitely, and soil microorganisms, most probably, were instrumental in nitrate filtration from the recharge water. The 1-year old seedlings and the calcareous sand recovered 42% of the nitrate of the NO_3^- enriched water, while taking NO_3^- concentration in floodwater and in zone 3 equal to 60.30 and 7.10 mg L^{-1} respectively, the 22-year old trees, the calcareous sand and, most probably, the vadose zone microorganisms retained 88.22% of the recharge water's dissolved nitrate. It should be emphasized that the maximum rooting depth of the 1-year old seedlings was 30 cm, while that of the trees in the afforested basins of the ARG systems was likely >23 m. Furthermore, the bench scale experiment lasted 12 hour on average, while the natural system functioned for many days. Thus, the eucalyptus roots provided an extremely large surface area in the vadose zone, filtering NO_3^- from the percolating and residual soil water for a much longer time. As Eucalypts consumes water copiously and the amount of rainfall and floodwater is not enough to saturate all vadose zone's profile during a flood event in the area, obtaining equilibrium seems impossible in the real conditions and needs further investigation. However, reducing nitrate uptake in that conditions might be possible as water consumption decreases as well.

According to Adams and Attiwill (1986ab) in their study on 8 eucalyptus forests, only one-third of N uptake was as NO_3^- . This conclusion was based on leaf analysis. In this study, NO_3^- was absorbed under strongly nitrifying conditions. Such a condition was available in zone 3, in which its p*E* was equal to 16.98, accompanied by the DO of 4.12 mg L^{-1}, on average.

The total N concentration of the leaves of 14 years old eucalyptus trees in the study area was 10.40 and 8.42 g kg^{-1} for the flood-irrigated and non-flooded basins, respectively (Mohammadnia et al., 2004). As flooding and non-flooding conditions were treatments in this case, the higher N content in the *Eucalyptus camaldulensis* Dehnh. trees growing in the flooded area was due to essentially absorption of more available NO_3^- from the vadose zone, and possibly converting it to other nitrogenous compounds in the leaves.

Consequently, purification of floodwater was intensified by the massive uptake of NO_3^- by the eucalyptus trees during the artificial recharge in the basins. Moreover, groundwater nitrate attenuation was also facilitated through the adsorption processes as well. Quantification of NO_3^- consumption by soil microorganisms requires another study.

CONCLUSIONS

This study evaluated the hypothesis that *Eucalyptus camaldulensis* Dehnh. and calcareous sand would filter NO_3^- from the recharge water. Floodwater NO_3^- was partially removed from the vadose zone of the ARG systems by the eucalyptus utilization and adsorption by the free $CaCO_3$ in the calcareous aquifer. Landuse and management practices significantly impacted groundwater NO_3^- ($P<0.01$), EC ($P<0.01$) and its pH ($P<0.05$) in the GBP. Obtained retardation factor (R) and the convection diffusion coefficients (D) of nitrate for eucalyptus planted columns confirmed mitigation of the dissolved NO_3^- from recharge flows as compared with non-planted columns in the saturated conditions of the soil of the recharge areas. As drainage of water ceases a few days after the recharge event, and there are very few recharge events during the year, it is logical to assume that most of the released NO_3^- is absorbed by the roots and not leached. We hypothesize that the CO_2 produced by rhizosphere respiration, which includes the respiration of living roots and of the microorganisms feeding on root-derived C (exudates and dead roots) (Rochette et al., 1999), is exchanged with the surface-bound NO_3^- in unsaturated conditions to supply the potential sites for nitrate adsorption in the coming recharge events. A detailed study under unsaturated conditions is required to confirm this hypothesis. In conclusion, establishing forested filter zones within the ARG systems facilitate a safer drinking water for rural inhabitants and desert-dwellers. Transformation of a marginal land into a prime agricultural land through floodwater-irrigated afforestation is the subject of another article.

As the costs of nitrogen inputs are currently one of the largest production costs in agriculture, nitrogen-enriched water is advantageous for irrigation farmers in this area-it reduces the need for N fertilization of the crops-deciding on the optimal proportion of the landscape devoted to purification of drinking water is the next phase of this study. This is a right move in achieving an eco-efficient agriculture. Although the high productivity and longevity of *Eucalyptus camaldulensis* Dehnh. are attractive attributes of this tree for its use in phytoremediation, and have large implications on soil and environment through carbon sequestration (Kowsar, 2005), the prodigious water consumption (Edraki et al., 2007) makes it undesirable in this water-short country. However, as our indigenous drought tolerant trees are very slow growing conifers and deciduous trees, and the latter are dormant during the rainy season, the eucalyptus offers the best choice at present. Therefore, we offer 2 practical solutions to this problem:

1. Selecting the fast growing, non-deciduous drought tolerant trees, which are as efficient as *Eucalyptus camaldulensis* Dehnh. in NO_3^-removal, but have a lower evapotranspiration requirement;
2. Decreasing the number of eucalyptus trees per unit area by thinning while maintaining the nitrate concentration below that of the USEPA's statutory maximum for drinking water. These entail further research.

As the first solution takes many years to give reliable results, we are working on both solutions simultaneously.

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