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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (1): 34-38, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.34.38

Effect of Sewage Irrigation on Nitrate Accumulation and Nitrate Reductase Activity in Leafy Vegetables

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Abstract: The effect of sewage water irrigation along with the N-fertilizers on NO_3 accumulation and on the *in vivo* NR assay in three leafy vegetable crops were studied. It was found that the NO_3 content in the leaves from the experimental sites were around two fold than the control and the NR enzyme activity was comparatively more in the control samples. The *in vivo* NRA with NaCl treatment increased at low level of NaCl where as with high level of NaCl, the enzyme activity decreased considerably. This is suggestive of the fact that NO_3 accumulation may be due to antagonistic effect of chloride ions.

Key words: Sewage, irrigation, salinity, nitrate reductase activity, N-fertilizers

INTRODUCTION

Agriculture is the major user of water and is able to accept even inferior quality water. In recent years, rapid increase in population and industrial growth has given rise to a water shortage due to limited water sources which, therefore, has forced "water reuse" to be one of the potential untapped water source. Sewage irrigation changes the chemical properties of the soil and also leads to accumulation of heavy metals in the edible parts of the crops (Sahu *et al*., 1992). In Mumbai, vast areas of land along the railway tracks are being utilized to cultivate several leafy and other vegetables almost throughout the year. These crops are irrigated with sewage water and also supplied with heavy doses of nitrogen fertilizers to gain quick yield and a better margin of profit.

Nitrate is usually the main source of N for many crop plants (Maynard *et al*., 1976). Both uptake and reduction are substrate inducible processes (Pilbeam and Kirkby, 1990). The NO_3 content in plants depends on the extent of nitrate uptake and assimilation. Of particular interest are the findings that when the supply of NO_3 is in the nutrient medium was high, the plants tend to accumulate NO_3 in their foliage tissue (Deane-Drummond, 1990). The increased nitrate content in plants may be the result of intensified nitrate uptake and/or decreased activity of NR. Besides, many studies have evaluated the nutrient concentration in the waste water which can be either beneficial as a valuable nutrient or be hazardous (Behera and Mishra, 1983; Misra *et al*., 1992). Considering these aspects, an investigation was conducted to study the effect of sewage water containing high amount of cations and anions in addition to heavy dose of N-fertilizer on NO_3 accumulation and enzyme NRA, of three leafy vegetable crops.

MATERIALS AND METHODS

The foliage of spinach (*Spinacea oleracea* L.), amaranth (*Amaranthus blitum* L.) and radish (*Raphanus sativus* L.) were collected from different sites selected, based on type of sewage water source. For comparison, these leafy vegetables were grown as control in micro plots supplied with optimum N-fertilizer for each and irrigated with tap water at the Botanic Garden of the institution. Leaves of the plants were harvested at harvest maturity between 8.30 to 9.30 h and employed for the sampling.

The total N content was determined by Kjeldahl method (Markham, 1942). Nitrate-nitrogen content in the leaves was determined Spectrophotometrically using Phenol-disulphonic acid as a reagent (Humphries, 1956) and the in vivo NR activity was determined in phosphate buffer pH 7.5 containing KNO_3 (Nicholas *et al*., 1976). Actual *in vivo* NR activity was measured as *in vivo* NR activity except that the incubation medium did not contain NO_3. The effect of variable concentration of NaCl on the *in vivo* NR activity was assayed in segments of leaves of plants grown as control. Prior to enzyme activity determination the segments were treated for two hours with different concentrations of NaCl (10, 50, 100 and 1000 mM) in nutrient medium.

The soil and sewage water samples were digested in the tri- acid mixture (Piper, 1950) and analyzed to estimate

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Na, K, Ca, Mg, P, Mn and Mo using Inductivity Coupled Plasma Atomic Emission Spectrometer (8440. Plasmalab). The chloride content from the soil sample (Volhard, 1956) and from water samples (APHA, AWWA and WPCF, 1980) was also determined.

RESULTS

Data obtained from the analysis of soils from different experimental locations including the garden soil as control (Table 1) showed mean Electrical Conductance (EC) values highest in soil irrigated by industrial water followed by industrial plus domestic sewage > tap water. This can be attributed to the concentration of ionic substances present in the soil. The concentration of N, NO_3-N, Ca, Mg, P, Fe, Mn, Cu and Cl were also found to be higher than the concentration found in the control soil, thereby clearly indicating the presence of excess amount of N, NO_3-N along with other elements. The percentage values for each element varied from one site to another and corresponded well with the type of sewer used for irrigation.

Data obtained from the water used for irrigation (Table 2) shows that the different sewers used for irrigation had comparatively higher concentration of divalent cations, monovalent cations and anions than the tap water, thus contributing to the total amount of different elements in the soil at all the sites. Similarly large increases in the total N, NO_3-N content were noted in the sewage over the control (tap water).These findings clearly indicate that the use of sewage water for irrigation has led to the increased concentration of different elements at the experimental sites than that found in garden soil (control).

In all the leafy vegetables, dry matter accumulation in the foliage tissue was maximum under domestic sewage water irrigation (Fig. 1). The content of total N in the foliage tissue increased when irrigated with sewage water irrigation and the highest content in each of the sample corresponded to the particular sewage water irrigation at which the accumulation of dry matter was the highest (Fig. 2). The nitrate level in the foliage tissue varied based on the type of sewage water irrigation and showed the following trend: industrial plus domestic sewage water > industrial sewage > domestic sewage water (Fig. 3). indicating an obstruction in primary assimilation of N.

Comparison of actual *in vivo* NR activity (minus NO_3) and the *in vivo* NR activity (plus NO_3) shows that NR activity supplemented with nitrate increased in all the excised tissue samples, however, in the experimental samples the differences were small clearly indicating that the nitrate availability was not rate limiting, whereas in the control, the differences were higher implying that NR activity was increasingly limited by lack of substrate (Table 3). A marked difference in the NR enzyme activity from each of the sample as influenced by the type of irrigation was observed (Fig. 4). Maximum activity was obtained in sample grown under tap water irrigation followed by domestic sewage>industrial sewage> industrial plus domestic sewage irrigation. These observations clearly imply that decrease in NR activity in the experimental samples was not due to limited supply of the substrate but attributed by the salinity in the external medium. Moreover, the decreased NR activity observed in plants under industrial plus domestic sewage irrigation was probably responsible for nitrate accumulation even though N uptake decreased.

Table 1: Analysis of Soil Sample form different experimental site

							Elemental concentration (%)									
Sample	Site	Organic (%) matter	Conductivity	Nitrogen (%)	NO_3-N (%)	pH	Na	K	Ca	Mg	P	Mn	Fe	Cu	Mo	Cl
Control		4.047	1.05	0.063	0.016	6.50	0.034	0.24	1.32	1.01	0.20	0.07	7.65	0.033	2.23×10^{-3}	0.102
Domestic	I	4.68	1.66	0.115	0.057	7.02	0.038	0.063	2.32	2.08	0.18	0.034	4.95	0.022	2.07×10^{-3}	0.199
sewage	II	5.30	1.51	0.102	0.0549	7.36	0.051	0.108	1.83	2.75	0.178	0.070	7.41	0.054	2.08×10^{-3}	0.184
Dom. + industrial	I	5.615	2.01	0.18	0.1273	6.80	0.049	0.152	3.93	3.67	0.243	0.092	11.04	0.033	2.83×10^{-3}	0.227
sewage	II	5.50	1.99	0.16	0.0875	6.77	0.041	0.199	2.99	1.69	0.248	0.065	8.17	0.053	1.03×10^{-3}	0.213
Industrial	I	4.77	3.08	0.13	0.098	7.40	0.074	0.094	1.76	1.24	0.21	0.074	9.8	0.044	2.40×10^{-3}	0.192
sewage	II	4.15	2.54	0.15	0.008	7.90	0.076	0.598	1.50	1.37	0.671	0.081	8.5	0.060	2.38×10^{-3}	0.017

Table 2: Analysis of Water Sample form different experimental site

					Elemental concentration (mg L^{-1})									
Sample	Site	Nitrogen (%)	NO_3-N (%)	pH	Na	K	Ca	Mg	P	Mn	Fe	Cu	Mo	Cl
Control		0.8	0.64	7.5	0.21	0.038	0.065	0.042	0.055	ND	ND	0.007	ND	0.39
Domestic sewage	I	84.2	22.10	6.2	18.36	8.72	7.56	2.73	0.39	ND	1.49	0.29	ND	74.04
	II	72.5	29.60	6.3	28.40	13.60	15.21	4.60	0.34	ND	2.83	0.37	ND	63.08
Dom. +industrial	I	90.3	32.50	5.6	45.50	20.80	29.20	9.80	3.42	ND	4.21	0.66	ND	347.05
sewage	II	122.0	59.80	5.4	97.64	23.20	32.70	7.40	4.03	ND	6.02	0.43	ND	166.07
Industrial	I	65.4	19.70	6.2	52.80	12.40	45.70	19.45	1.24	ND	1.19	0.37	ND	99.03
sewage	II	52.7	20.50	6.8	13.30	3.36	88.08	30.24	1.45	ND	1.98	0.26	ND	102.08

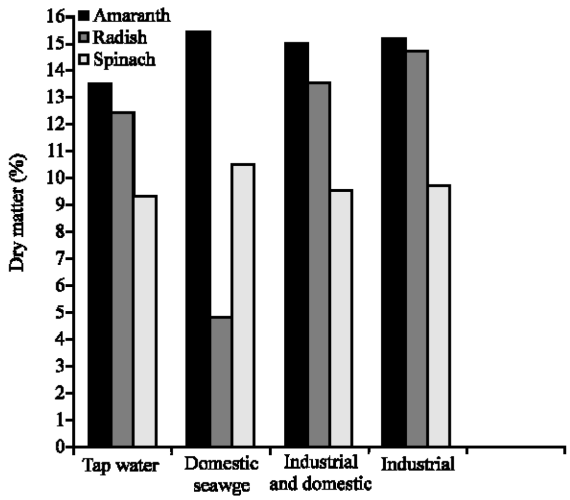

Fig. 1: Effect of types of irrigation on percent dry matter in the leaves of spinach, radish and amaranth

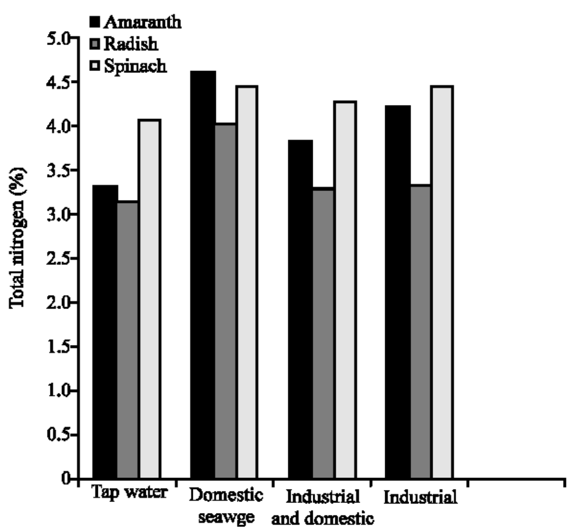

Fig. 2: Effect of types of irrigation on percent total Nitrogen in the leaves of Spinach, radish and amaranth

Table 3: Comparison of Actual *in vivo* (-NO_3) and *in vivo* (+NO_3)

Type of irrigation	Plants	NRA (µ mol NO_2 g h^{-1}) Actual *in vivo* (-NO_3)	*In vivo* (+NO_3)
Control	Amaranth	2.15	4.38
	Spinach	0.996	2.46
	Radish	2.01	5.92
Domestic sewage	Amaranth	3.27	3.98
	Spinach	0.806	1.05
	Radish	4.73	5.21
Dom. + industrial sewage	Amaranth	1.21	1.26
	Spinach	0.246	0.29
	Radish	2.38	2.78
Industrial sewage	Amaranth	2.11	2.96
	Spinach	0.59	0.73
	Radish	3.163	3.318

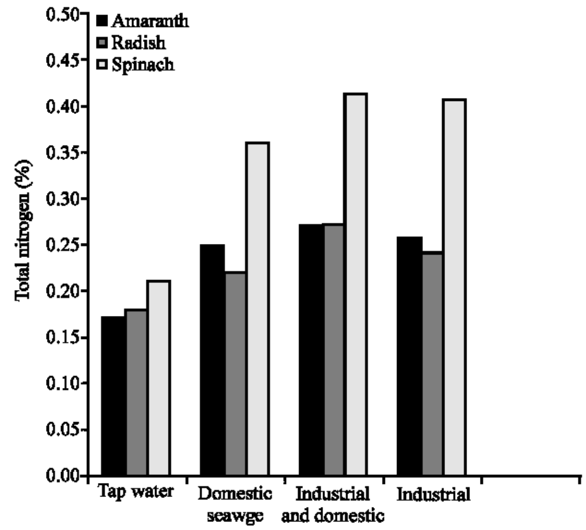

Fig. 3: Effect of types of irrigation on percent nitrate-nitrogen in the leaves of Spinach, Radish and Amaranth

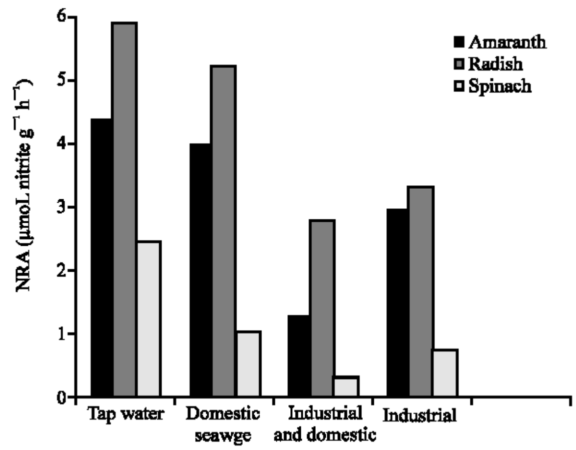

Fig. 4: Effect of types of irrigation in addition to Nitrogen fertilizers on the *in vivo* NRA

The *in vivo* NR activity from NaCl treated tissues was determined. The response of all the foliage samples to 10 mM concentration of NaCl was similar and increased around 60% over untreated samples. The enzyme activity in amaranth and radish foliage increased even at 50 mM concentration of NaCl, though further increase in NaCl concentration reduced NR activity. In spinach, however the NR activity decreased beyond 10 mM concentration of NaCl. At the 1000 mM concentration the enzyme activity decreased considerably around 20-50% over untreated samples (Fig. 5). Nitrate accumulation, though a natural phenomenon, is also influenced by external supply of nutrients besides nitrate (Ohta *et al*., 1987, 1988). An abundant supply of potassium and sodium are

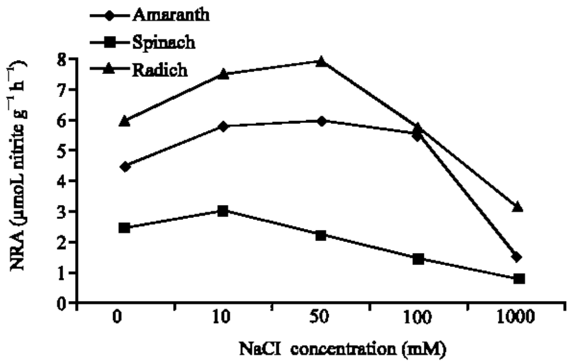

Fig. 5: Effect of NaCl (mM) concentration on the *in vivo* NRA

said to stimulate nitrate consumption by plants, while calcium is known to affect nitrate uptake and its reduction by plants (Haynes and Goh, 1978; Rao and Rains, 1976). The uptake of nitrate appears to be influenced by anions such as chloride. On basis of the data obtained (Table 1, 2) it is reasonable to assume that the high accumulation of nitrate observed in the foliage tissue of the plants grown under sewage irrigation is aided by the presence of excess amount of nitrate-nitrogen along with other nutrient elements in the soil solution.

DISCUSSION

NR is generally accepted as a rate limiting enzyme in the assimilation of nitrate by higher plants and Nitrate has been shown to induce NR (Clarkson and Deane-Drummond, 1983). The increased content of nitrate despite the lower content of total N in these plants under the sewage water irrigation may be either due to the failure of nitrate nitrogen level in the tissue to induce NR enzyme or due to the low activity of the necessary enzymatic system. The decrease in the activity of NR in presence of high NaCl concentration observed is in conformity with the previous findings (Ramasubramaniam *et al*., 1993). NR belongs to a group of inductive enzymes thus the decrease in activity at high level of NaCl might be a reflection of either a decrease or an increase of nitrate uptake. The other possibility is that chlorides and nitrate ions are mutually antagonistic, thus excess of chloride ions may cause nitrate substrate deficit for the enzyme induction (Cram, 1973). It has been reported that chloride inhibition resulted from the formation of an inactive ternary complex as the chloride ions bind weakly to the NR-NO_3 complex. Such bindings have been reported to weaken the binding of NO_3 to the enzyme. Besides, a direct ligation of the chlorides to the Mo component of the enzyme NR is another suggested possible inhibitory mechanism (Barber *et al*., 1989). Thus, excess of sodium chloride affects the activity of NR. The responsive behavior of NR to metabolic and physiological status of plants has been reported earlier and thus NR is being used as an indicator of plant stress (Lorenzo *et al*., 2001). Excessive salinity affects number of metabolic processes in plants including N- assimilation. The negative effect of NaCl on plant N- assimilation is strictly related to salt stress induced modification of the enzymes involved in Nitrate assimilation pathway (Mores *et al*., 2004; Debuba *et al*., 2006). Recently, Bybordi *et al*. (2010) have studied the combined effect of salinity and the nitrogen source type added to the nutrient solution on productivity, photosynthesis and nitrogen metabolism and reported NRA to be significantly decreased by salinity stress treatment.

In conclusion, based on our data and from the pertinent previous study, it is reasonable to assume that increase in leaf nitrate content in the leaves of the plants irrigated with sewage water is mainly due to the high nitrate content in the soil solution. Enzyme NR activity decreased in the foliage of the plants grown in the experimental sites whereas the leaf nitrate content increased. Thus, indicating that the nitrate is not the limiting factor for the enzyme induction resulting in the decrease in enzyme activity but the salinity in the external medium lowered the activity may be due to enzyme degradation or the reduction in gene expression and nitrate reductase protein synthesis.

ACKNOWLEDGMENT

The authors are thankful to Prof K.V. Hippalgaonkar for the guidance rendered during the experimental work.

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