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INFLUENCE OF NITROGEN NUTRITION ON THE IONIC BALANCE OF MAIZE GROWN IN CALCAREOUS SOIL

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ABSTRACT

The objectives of this study were to examine the influence of nitrate, ammonium and ammonium + N-Serve on ionic balance and N uptake by maize plants. Maize plant were grown in calcareous soil supplied with 100 and 200 mg N kg^{-1} soil in the forms of KNO_3, $(NH_4)_2SO_4$ (AS) and $(NH_4)_2SO_4$ + Serve (AS+NS) and sampled at 30 and 60 day after sowing (DAS). Results revealed that at different growth stages, the combination of ammonium sulfate with nitrapyrine (N – Serve) gave the highest value of dry matter and N uptake as compared with other treatments. Application of N – Serve with ammonium sulfate resulted in decreasing the accumulation of NO^-_3 in maize leaves. NO^-_3 concentration was higher at 60 DAS than that found at 30 DAS and at 200 mg N/kg soil than 100 mg N/kg soil except when N–Serve was applied. Ratio of (K^+/Ca^{2+} + Mg^{2+}) was constant with all treatments which increased with ammonium sulfate + N–Serve treatments (AS + NS). Total uptake of inorganic cations and anions were determined by analysis of tissue for K^+, Ca^{2+}, Mg^{2+}, Na^+, total N, NO^-_3, $SO^=_4$, $PO^=_4$ and Cl^-. Differences in total inorganic cations (C) and anions (A) in plant tissue were used to estimate total carboxylate (C-A). Internal OH^- generation resulting from excess cation uptake (net H^+ excretion) by the roots. The (C-A) of plant supplied 100 mg N/kg soil ranged from 85 to 116 at 30 DAS and 130 to 149 at 60 DAS. The increments in nitrogen levels tended to increase total cation uptake especially, with plants fed with ammonium + N-Serve. The increase in cation uptake was paralleled by an increase in anions uptake. The C to A uptake ratio for plants supplied with 200 mg / kg soil ranged from 2.9 to 3.4 at 30 and 60 DAS, respectively, indicating that the internal OH^- was generated both by excess of cation uptake and by NO^-_3 and $SO^=_4$ reduction. On a seasonal basis, only 15 % of the OH^- generated during NO^-_3 and $SO^=_4$ reduction was associated with OH^- efflux (excess anion uptake), and 85 % was associated with carboxylate accumulation.

Keywords: carboxylate (C-A), N-Serve (NS), Ammonium Sulphate, Nitrate accumulation.

INTRODUCTION

Maize is the most widely cultivated cereal crop in the world after wheat and rice. Proper management of N fertilizer in calcareous soils involves practices that minimize its loss through ammonia volatilization. Regarding NH_3 losses from urea, additional practices include mixing the urea with $CaCl_2$ or TSP and use of granular forms, urase inhibitors and sulfur-coated urea (Wiezler, 1998).Abd El-Galil (2000) and Hellal *et al.*, (2006) studied the effect of nitrogen supply in combination with nitrification inhibitors for wheat and maize crops grown in alluvial and calcareous soil. They concluded that, at harvest and different growth stages the combination of ammonium sulfate with Nitraprine gave the highest dry weight and had a prominent effect on nitrogen and iron uptake and chlorophyll content and minimizing nitrate accumulation.

When NO^-_3 is supplied in the nutrient medium, as is the normal case for plants growing in soil, the situation is different. Anion uptake exceeds cation in comparison with other forms of N nutrition; higher concentrations of organic acids are accumulated in NO^-_3 – fed plants (Raven and Smith, 1976). With increasing NO^-_3 nutrition the bulk of the anion charge appeared as organic anion accumulation in the plants and OH^- efflux into the nutrient solutions as calculated by anion than cation uptake. The increase in organic anion accumulation was paralleled by an increase in cation concentration (K^+, Ca^{2+}, Mg^{2+} and Na^+). Total inorganic anion levels (NO^-_3, $SO^=_4$, $H_2PO^=_4$ and Cl^-) were relatively constant (Ernest and Alistair, 1977)

The plants that produce large quantities of organic acid have greater cation: anion uptake ratio and they generate more soil acidity than plant with a small cation : anion ratio. The form of N absorbed by plant may also affect the cation: anion uptake ratio (Jarvis and Robson, 1983). Regardless of whether N is absorbed as NH_4, NO_3 or N_2 (N fixation) the excess cation content often calculated from the nitrogenous cation and anion (Monaghan *et al.*, 1998) this because it is difficult to establish the form of nitrogen absorbed.

The biochemical processes of plants causing acidification have been reviewed by Bolan *et al.*, (1991) and DeKlein *et al.*, (1997) during photosynthesis organic acids are produced and the cytoblasmic pH of plants (7.2-7.4), some of carboxyl groups of this acid this dissociated to release H^+ ions. Cation uptake by plants results in excretion of this H^+ ions into the soil solution to maintain charges neutrality. Since, OH^- or HCO_3^+ are excreted during uptake of anion, soil acidification only occurs when cation uptake exceeds anion uptake.

The objective of this study was to examine the influence of N- level and N- source and preventing the nitrification of soil applied NH^+_4 forcing the plant to take up NH^+_4 and changing in the nutrient soil balance, uptake and transport processes of maize plants.

MATERIALS AND METHODS

A greenhouse experiment was conducted at National Research Centre, to evaluate the influence of level and source of nitrogen on Cation-Anion balance of corn plant. The soil used was a calcareous soil characterized by $CaCO_3$ 10.8 %, pH 8.11, EC 1.13 dS m^{-1} and organic matter 1.05 %. Pots were filled with ten Kg of air dried soil. Two level nitrogen (100 and 200 mg N / kg dry soil were applied as KNO_3, $(NH_4)_2SO_4$ or $(NH_4)_2SO_4$ + N–Serve (Nitrapyrin). The nitrapyrin was added as 1 % of N applied added after sowing. All pots received 3.8 and 0.85 g pot^{-1} from superphosphate and potassium sulphate as source of P and K, respectively. Sufficient amount of micronutrients applied before sowing.

The experiment was laid out in randomized complete block design with three replicates. Maize (*cv.* Giza hybrid 321) was germinated and five uniform seedlings were planted in each pot and irrigated in away to maintain the soil moisture at 60 % of the water holding capacity a long the growing season. Plant samples were taken at 30 and 60 day after sowing (DAS), fresh and dry weights of plant were recorded. The plant was harvested, washed thoroughly with tap water, rinsed with 0.1 HCl for one minute and then washed with distilled water.

The plant dried at 70 °C and grinded for analysis. Nitrate nitrogen was determined in fresh leaves using method described by Cottenie *et al.*, (1982). The digested plant solutions were analyzed for N using micro Kjeldahl technique and P determined colormetrically using spectrophotometer (Jaiswal, 2003). K and Na were determined using Flamephotometer. Sulphur determined colormetically after wet digesion with HNO_3 and $HClO_4$ according the method described by FAO, 1970). $Ca^{++}$and Mg^{++} content in plant samples were determined using acid digestion technique and measured in an atomic absorption spectrophotometer. Cl^- content in the plant samples were determined according to method described by Cottenie *et al.*, (1982). Total uptake of inorganic cations and anions was determined by analysis of tissue for K^+, Ca^{2+}, Mg^{2+} and Na^+, total N , NO^-_3 , $PO^=_4$, $SO^=_4$ and Cl^-. Differences in total inorganic cations (C) and inorganic anions (A) in plant tissue were used to estimate total carboxylate content. Obtained data were subjected to statistical analysis according to Dumcan's multiple range test and determined the LSD test at 5%.

RESULTS AND DISCUSSION

Dry matter Production

Dry matters of two ages of maize plant for the different treatments are given in Table (1). As expected, increasing the level of nitrogen supply produced a very marked influence. Dry matter particularly at the higher level of nitrogen (200 mg/kg) treatments was about 1.2 times grater than in the lowest nitrogen level (100 mg/kg). The addition of N – Serve with 200 ppm ammonium sulphate (AS) resulted in a significant increase in dry matter weight by 22 and 13.6% at 30 and 60 days over potassium nitrate treatment and was superior over ammonium sulphate alone without N – Serve. Similar effect with potassium nitrate and ammonium sulfate without N – Serve were also observed. These results are in good agreement with those obtained by Blackmer and Sanchez (1988).

Nitrogen uptake

The influence of levels and different sources of nitrogen on nitrogen uptake are shown in Table (1). Results noticed that, increasing the level of nitrogen from 100 to 200 ppm produced a dramatic effect on the N uptake by the growing plant. However, the uptake of nitrogen was greatly increased by different N treatments. Application of $(NH_4)_2SO_4$ + NS had a predominant effect on the N uptake by maize plant as compared to $(NH_4)_2SO_4$ alone or KNO_3. Nitrification inhibitor treatments [$(NH4)_2SO_4$ + NS] showed higher values of N – uptake at 30 and 60 days after sowing reaching (518 and 1057 mg/pot) at lower N level (100 ppm N), respectively. The combination between NS (N-serve) and higher levels of AS gradually increased the N uptake by 33.6 and 28 % as compared with application of KNO_3 at both growth stages.

Table (1): Influence of N sources, levels and plant age on growth and nitrogen uptake by maize plant.

N sources	N levels (ppm)	Plant age (days)	Dry weight (g pot^{-1})	N uptake (mg pot^{-1})	NO^-_3 (ppm)
KNO_3	100	30	13.8	429	2081
		60	31	826	3037
	200	30	16.7	539	2413
		60	33	883	3298
$(NH_4)_2SO_4$	100	30	13.3	376	1903
		60	32.5	809	2530
	200	30	15	449	2014
		60	36	906	2970
$(NH_4)_2SO_4$ + N-Serve	100	30	14.6	518	1571
		60	35.4	1057	1895
	200	30	18.3	720	1749
		60	37.5	1131	2442
LSD 5%			1.54	3.15	55

The combination between NS and Lower levels of AS increased the N uptake by 37.7 and 30.6 % at both growth stages. N uptake by plants treated with AS + NS or KNO_3 was higher than the plant treated with AS alone. It could be assumed that the addition of nitrification inhibitor helped in providing more nitrogen in the form of NH^+_4 which might be preferred by young plant. Plant can absorb nitrogen in both forms (NH^+_4 and NO^-_3), old plants can accumulate and reduce absorbed nitrate and use it in metabolism with the same efficiency using NH^+_4 form. The results in consonance with the finding of Hellal *et al.* (2006).

Nitrate accumulation

As regards nitrate accumulation in maize plant, it is clear from the data in Table (1) that nitrate accumulation varied due to different treatments and the level of nitrogen at both stages. Results indicated that nitrate concentration in maize plant was markedly decreased by using N – serve particularly at second stage as comparing other treatments in both stages. However, the nitrate content (60 days) was higher than that at first stage (30 days). This phenomenon could be due to the oxidation of NH^+_4 to NO^-_3 from through the nitrification process creating higher NO^-_3 accumulation in plant tissues. The accumulation of nitrate significantly increased by the application of potassium nitrate levels by about 60 and 35 % over AS + NS treatments at 100 and 200 ppm at 60 days, respectively.

Ruminants are subjected to nitrate poisoning (methemoglobinemia) when forage nitrate exceeds 0.9 to 1.8 %, of though nitrate as low as 0.6 % also have induced aborption in cattle and sheep. Although nitrate percentage high enough to cause methomoglobinernia (Edwards and McCoy, 1980). Whereas, application of N – Serve with AS at two levels of nitrogen reduced the NO_3 content in the plant tissues as compared with other treatments. This could be due to the application of NS effectively depressed the oxidation of NH^+_4 by Nitrosomonas bacteria and can therefore, increase the effectiveness of NH^+_4 applications in reducing the nitrate content of lettuce (McCall and Willumsen, 1998). Increased nitrogen level from 100 to 200 ppm significantly increase NO^-_3 concentration by 8.5, 17.3 and 28.8 % at 60 DAS with KNO_3, $(NH_4)_2SO_4$ and $(NH_4)_2SO_4$ + NS, respectively.

Cations and Anions uptake

Data in Table (2) represent the uptake of K, Ca, Mg and Na by maize plant at two growing stages under study. The content of these cations in plant material seemed to be affected by adopted treatments.

Table (2): Effect of N sources and levels on cation uptake (meq 100 g^{-1}) by maize plant.

N sources	N levels (ppm)	Plant age (days)	K^+	Ca^{2+}	Mg^{2+}	Na^+	$\frac{K}{Ca + Mg}$
KNO_3	100	30	55	43.5	28.9	9.4	0.75
		60	70	62	45.6	17	0.69
	200	30	60	48	41	11	0.67
		60	71	64.6	41.3	17	0.56
$(NH_4)_2SO_4$	100	30	50	38	30	9	0.73
		60	82.7	67	54.8	19.5	0.66
	200	30	57.0	47	39	10	0.66
		60	102	78	55	20.6	0.85
$(NH_4)_2SO_4$ + N-Serve	100	30	63.0	48	43	10	0.70
		60	89	63.7	56.8	4	0.73
	200	30	68	52	43	11	0.71
		60	97	70	50	17	0.84

LSD at 5% for potassium divided by sum of Calcium + Magnesium is equal to 0.044

Potassium concentration was higher in the last stage (60 DAS) than the first stage (30 DAS) in all treatments. Increasing nitrogen application tended to increase the K concentration. The inhibitor did not affect on the pattern of K absorption, since differences in K concentration and uptake between the two N applications were narrow. Lower K uptake in N– serve treatment was noticed in two stages at two levels of nitrogen as compared with no inhibitor treatments. Therefore, it could be stated that the inhibitor though increased dry matter production but decreased K absorption. This might be attributed to the possible competition between NH^+_4 and K^+ ions. The negative effect could be noticed also for the concentration and uptake of Ca and Mg. Cox and Reisenouer (1977) reported that intake of mineral cations in a dilute constant composition in water culture, decreased by added NH^+_4 , ammonium had a greater effect on the intake of divalent than of the monovalent cations. It has been reported that the Ratio of (K/Ca + Mg) in forage has a special significance. A ratio of more than 2.2 is considered hazardous and might cause grass tetany (Mathers *et al.*, 1989), the maize in the first stages used forage for animal.

Grass tetany (a form of by pomog nesmic tetany) occurs when there is a low level of magnesium (Mg) in the cow's blood. Crass tetany, is caused by a low content of Mg in pasture or hay. The availability of Mg is reduced if the forage is high nitrogen (N) or potassium (K).

The calculated ratio of K/ (Ca +Mg) under this study never reached the critical limit even in presence of the inhibitor. The cations uptake increased with increase the level of nitrogen. The use of nitrification inhibitor might decrease the concentration of Ca and Mg more than of K and cause such increase (0.84) which induces tetany.

The influence of nitrogen application levels and the age of plant on anion uptake shown in Tables (3). For all treatment, the cation uptake exceeded anion uptake by 18.0 and 27 % for plants supplied 100 and 200 mg kg^{-1} at 60 DAS after sowing, indicating a considerable net H^+ efflux from the roots.

Table (3): Effect of N sources and levels on anion uptake (meq 100 g^{-1}) by maize plant.

N sources	N levels (ppm)	Plant age (days)	NO^-_3	$SO^=_4$	$PO^=_4$	Cl^-
KNO_3	100	30	3.4	16	17	3.4
		60	11.5	22	26.8	4.5
	200	30	4	21.4	19.2	3.5
		60	10.7	26.7	25	4.2
$(NH_4)_2SO_4$	100	30	3	19	17	3.3
		60	10.5	28.5	30.8	4.8
	200	30	3.5	19.8	18	3.9
		60	12	37	32	5.8
$(NH_4)_2SO_4$ + N-Serve	100	30	2.7	22	20	3.4
		60	6.7	35.6	32.8	5.7
	200	30	2.9	21.5	21	3.3
		60	9	33.8	28	4.9

A primary mechanism by which plants cause acidification is by releasing protons into the soil when cations uptake exceeds anions uptake (Tang and Rengel, 2003). The cations to anions uptake ratio ranged from 2.83 and 3.10 for $(NH_4)_2SO_4$ and AS + NS treatment at 60 DAS. The highest cations content occurred in the $(NH_4)_2SO_4$ + NS plants and was lowest in the nitrate and ammonium without N-serve treatments. Sulfate and $PO^=_4$ were the major absorbed by AS + NS plants. Whereas, Nitrate was the major anion absorbed by NO_3 dependant plants.

Excess cation uptake increase by 1.39 and 1.35 times at 60 DAS than 30 DAS of growth, and the same trend was noticed with the total anions. (Curtin and Wen, 2004) indicated that the total cations (Ca + Mg + K + Na) in Kochia plants exceeded those of anions (Cl^- + S + P + NO^-_3) by 250 to 280. At all treatment K^+ + Ca^{++} uptake comprised more than 70 % of total cation uptake by plants. Regardless of the N source , K^+ uptake exceeded Ca^{2+} and Mg^{2+} uptake but the ratio of K^+ to Ca^{2+} uptake was lower for KNO_3 treatment (1.1) than AS+NS and AS (1.38 – 1.30), respectively at 60 DAS with 200 mg N kg^{-1}. Similarly were noticed with KNO_3 and $(NH_4)_2SO_4$ treatment.

The cations to anions uptake ratio in Table (4) for the plants applied 10 millimolar NO^-_3 ranged from 1.3 to 1.57 during development, indicating that internal OH^- was generated both by excess cation uptake and by NO^-_3

and $SO^{=}_4$ reduction (Daniel et al., 1982). Differences in total inorganic cations (C) and inorganic anions (A) in plant tissues were used to estimate total carboxylate content. Reduction of NO^-_3 and $SO^{=}_4$ as well as great uptake of inorganic cations than that inorganic anions, contributes to the accumulation of excess inorganic cations (C – A) and to the generation of OH^- within the tissue.

Table (4) : Influence of nitrogen sources and levels on total uptake of cations (C) and anions (A) by maize plants.

N sources	N levels (ppm)	Plant age (days)	Cations (C)	Anions (A)	C-A	C/A
KNO_3	100	30	136.8	39.8	97	3.4
		60	194.6	64.8	130	3.0
	200	30	160	48	112	3.3
		60	194	66.6	127	2.9
$(NH_4)_2SO_4$	100	30	127	42.3	85	3.0
		60	224	74.6	149	3.0
	200	30	153	45	108	3.4
		60	255	86.8	168	2.82
$(NH_4)_2SO_4$ + N-Serve	100	30	164	48	116	3.4
		60	228	80.8	147	2.93
	200	30	174	51	123	3.4
		60	234	75.7	158	3.09
LSD at 5%			2.32	1.5	2.99	0.18

C – A= Excess cation uptake (carboxylate), C/A= Cation / Anion uptake

For plant assimilating $SO^{=}_4$ and N–serve in the absence of the readly permeable NO^-_3 anion, inorganic cation uptake (17 and 22.6) exceeds inorganic anions uptake substantially resulting in acidification in the root zone. The quantity of carboxylate (C – A) in tissue of plant supplied NH^+_4 than was only a small fraction of that observed for NO^-_3 supplied plants and most of the H^+ produced were during assimilation of NH^+_4 (Raven and Smith, 1976). The proportion of total carboxylate accumulation associated with internal OH^- generated by excess cation uptake increased from 106.8 to 146.5 as the plants aged at 60 day old and C/A ratio declined from 3.0 to 1.5 by increased the nitrogen application from 100 to 200 mg/pot with nitrate treatments. The anion change which balances excess cation uptake is presumably converted from the OH^- to carboxylate

Correlation coefficients

Simple correlation coefficients of different estimated nitrogen parameters of maize plants are shown in Table 5.

The N uptake showed very high positive significant correlation with cation (0.907**), anions (0.899**) uptake and Carboxylate release (0.897**) and negative correlation with C/A. This indicates that the importance of N uptake for marinating the cations and anions balance of maize plants leading to increase in growth and yield. The release of HCO^-_3 or OH^- (C-A) had highly positive significant correlation with dry weight, nitrogen uptake, NO^-_3 accumulation and cation and anion uptake by maize plants. The ratios of K/(Ca+Mg) was positively and significantly correlated with total uptake of cation (0.341*) and C-A (0.367*). This indicates the importance of K/ Ca + Mg for maize plants in cation uptake balance and inducing the tetany appear on maize plants.

Table (5): Correlation analysis between nitrogen parameters and ionic ratios of maize plants.

	Dry wt.	N uptke	NO_3^-	Cations	Anions	C/A	C-A
N uptake	0.939**						
NO_3^-	0.641**	0.440**					
Cations	0.939**	0.907**	0.476**				
Anions	0.962**	0.899**	0.522**	0.979**			
C/A	-0.831**	-0.817**	-0.505**	-0.753**	-0.808**		
C-A	0.902**	0.897**	0.431**	0.987**	0.939**	-0.695**	
K/(Ca+Mg)	0.201	0.255	-0.179	0.341*	0.298	-0.282	0.367*

** Significant at 1% , * Significant at 5% , C: cations, A: anions and C-A= Carboxylate ions

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تـــأثيراضافة ا لنيتروجين على الاتزان الايونى لنباتات الذره الشـــاميه المنزرعـــه فى ارض جيريه

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تهدف الدراسة إلي اختبار تأثير التغذية الازوتية (النتراتية ، الامونيومية والامونيومية + مثبط التأزت) علي الاتزان الأيوني وامتصاص النيتروجين بواسطة نبات الذرة الشاميه .
في تجربة صوبة تم زراعة الذرة الشاميه في أرض جيرية أضيف إليها أسمدة ازوتية بمستويات ١٠٠ و ٢٠٠ مليجرام نيتروجين / كجم تربة في صورة نترات البوتاسيوم، كبريتات الأمونيوم و كبريتات الأمونيوم + مثبط التأزت (N- serve) وتم حصد النباتات بعد ٣٠ و ٦٠ يوم من الزراعة . الامتصاص الكلي لكل من الكاتيونات (C) والانيونات (A) قدر بتحليل كل من البوتاسيوم ، الصوديوم ، الكالسيوم والماغنسيوم وأيضا النيتروجين الكلي ، النترات ، الكبريتات ، الفوسفات و الكلوريد – والاختلاف بين الكاتيونات الكلية (C) والانيونات الكلية (A) في الأنسجة النباتية استخدام لتقدير الكربوكسيل الكلي (C-A) .

وأوضحت النتائج ما يلي :

- أعطت معاملة كبريتات الأمونيوم + مثبط التأزت اعلي قيم لمحصول المادة الجافة وكذلك امتصاص النيتروجين عند المراحل المختلفة للذرة عند المقارنة بباقي المعاملات .
- حدث انخفاض في كمية النترات المتراكمة في أوراق نباتات الذرة نتيجة لإضافة مثبط التأزت إلى كبريتات الأمونيوم .
- كان تركيز النترات في الانسجة النباتية اعلي عند ٦٠ يوم عنه عند ٣٠ يوم من الزراعة وأيضا عند إضافة ٢٠٠ مليجرام عنه عند إضافة ١٠٠ مليجرام نيتروجين / كجم باستثناء معاملة كبريتات الأمونيوم + مثبط التأزت .
- القيم النسبية للبوتاسيوم مقسوما على مجموع الكالسيوم والماغنسيوم كانت ثابتة مع كل المعاملات المدروسة ولكنها زادت مع المعاملة المضاف إليها مثبط التأزت .
- كان هناك انتاج عال لأيونات الهيدروكسيل (OH^-) نتيجة لزيادة امتصاص الكاتيونات (انطلاق هيدروجين H^+) بواسطة الجذور .
- قيم (C - A) في النباتات المسمدة لـ ١٠٠ مليجرام نيتروجين / كجم تربة تراوحت من ٨٥ – ١١٦ بعد ٣٠ يوم من الزراعة ومن ١٣٠ – ١٤٩ بعد ٦٠ يوم من الزراعة .
- زيادة مستويات التسميد الازوتي ادت إلى زيادة الكاتيونات الكلية الممتصة بواسطة النباتات وخصوصا عند التغذية بمعاملة كبريتات الأمونيوم + مثبط التأزت .
- كانت معدلات الزيادة في امتصاص الكاتيونات تتساوى مع معدلات الزيادة في امتصاص الانيونات ، وكانت نسبة امتصاص الكاتيونات : الانيونات للنباتات المسمدة بمستويات عالية من الازوت تتراوح من ٢,٩ – ٣,٤ بعد ٣٠ و ٦٠ يوم من الزراعة علي التوالي وهذا يوضح أن هناك انتاج عالي للهيدروكسيل (OH^-) نتيجة لزيادة امتصاص الكاتيونات وأيضا اختزال كل من النترات والكبريتات .
- كانت ١٥ % فقط من (OH^-) المنتج أثناء اختزال كل من النترات والكبريتات تتزامن مع OH^- efflux (زيادة امتصاص الانيونات) بينما ٨٥ % منه كانت مصاحبة لتراكم الكربوكسيل .