Halophytes, the answer to all your salt problems

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Abstract
Accumulation of salt in wastewater and soil is a increasing environmental and economic problem that might be solved with use of halophytes. To do so understanding of how halophytes take up and distribute sodium is needed. Also more insight on the use of halophytes as agricultural products is important.
With this information, an economically valid and reliable system of removing salts from wastewater may be build. The halophytes take up salt through the roots and distribute it by the active pumping of the sodium ions into the vacuoles. The ability of halophytes to grow in a high saline surrounding makes them useful as a guard against saline pollution to the environment. The halophytes can have a double use, as they can also be used as crops for the production of oil seeds and biofuel.
The use of halophytes is recommended for the desalination of waste water at an industrial site near A.G. Wildervanckkanaal.

Table of contents

Introduction

In the process of water purification, in which water with low salt contents is being produced, water with very high salt contents is produced as waste product. High salt concentrations can have very negative consequences for the environment.
Soil salinity is a serious environmental hazard in agriculture. It limits crop yield and restricts the use of land previously cultivated. Around 20% of the world's agricultural land and nearly half of all irrigated land is affected by soil salinity. Agriculture sees more and more problems because of soil salinization due to irrigation.[1] One of the principal adverse effects of high salinity in non-tolerant plants is growth inhibition by toxicity to Na.

In saline conditions, sodium ions and potassium ions compete with each other for uptake through common transport systems and this happens often, since in the environment the concentration of sodium ions is usually higher than that of potassium ions.
A high sodium : potassium ratio, exerts metabolic toxicity by competition between sodium-, and potassium- ions for the binding sites of many enzymes. [2]
Therefore saline waste water cannot simply be dumped into the environment. There are chemical ways to get rid of salt such as using gypsum to neutralize the sodium ions by having it bind to the sulphate, but this is labour intensive and expensive.[3] Biological ways to reduce the salt contents of this water may constitute a useful contribution. To address this question it may be useful to examine the use of halophyte plants.

Halophytes represent only two percent of terrestrial plant species. They are present in about half the higher plant families and represent a wide diversity of plant forms. All halophytes seem to have evolved the same basic method of osmotic adjustment: accumulation of inorganic salts, mainly sodium chloride, in the vacuole and accumulation of organic solutes in the cytoplasm. Gradually we obtain better insight into the differences between halophyte and glycophyte ion transport systems. The pathways by which sodium and chloride ions enter halophyte cells, however, are still not fully understood. These pathways may involve ion channels and pinocytosis, in addition to sodium and chloride transporters.[4]

Uptake and distribution of sodium in halophytes

The protection of sodium-sensitive metabolic mechanism under saline conditions partly depends on the ability to keep the levels of sodium ions in the cytosol low. The most important way that plant cells keep the sodium concentrations in the cytosol low, is to minimize the influx and maximize the efflux of sodium from the cytosol. The sodium ions are either moved into the apoplast or into the vacuole. One of the ways that plant cells could restrict the influx of sodium is by selective ion uptake. Several different types of cation channels seem to be involved in mediating the influx of sodium ions, these channels include outward- and inward-rectifying potassium selective channels and non-selective cation channels (NSCCs). High affinity potassium transporters have also been

believed to mediate considerable sodium ion entry into the plant roots. Recent findings however, suggest that the dominant pathways for sodium ion influx into root cells are the non-selective cation channels. The effectiveness of these channels, however, are inhibited by the presence of larger amounts of calcium ions.[5]

Halophytes drive water into the plant against a low external water potential by using the controlled uptake of sodium ions into the cell vacuoles (balanced by chloride ions and other anions).

There are diverse opinions about the mechanisms of sodium entry into halophyte cell tissues.
In glycophytes, two type of leakage are thought to be responsible for the entry of sodium ions into the plant. In some plants, large quantities of water enter into the plants, via transpirational bypass flow: Water travels through the root in extracellular spaces rather than in the symplasm. Thus, the water bypasses the endodermis to enter the transpiration stream directly.

If there is an excess of sodium chloride in the external solution, enough sodium ions can be carried to the shoot via bypass flow, to poison the leaves. In other plants, such as wheat, the transpiration bypass flow may be low. But in that case the sodium ions leak into the plant via the symplasm of root cortical cells. It does so by competitive binding onto the transporters of potassium ions or onto cation channels. Again, such leakage allows damaging levels of sodium ions to enter the plant. At the same time it also depresses the uptake of potassium ions.

In halophytes, these mechanisms do not seem plausible, at least with respect to the entry of sodium ions. Halophytes can often prevent transpiration bypass flow because most halophytes have thick layers of suberin or double layers of suberized cells at the root endodermis cells. These layers or cells, only allow the uptake of sodium chloride through the symplasm.
There does not seem to be a relation between the cellular uptake of sodium and potassium ions in halophytes. Halophytes tend to maintain steady rates of potassium ion uptake across wide ranges of external sodium concentrations and rate of sodium uptake. It seems unlikely that sodium ions enter into halophyte cells through potassium-ion carriers.

In some halophytes the uptake of sodium ions by the roots is greater than the uptake potassium ions through the same route. The sodium uptake is probably too rapid for known carrier transport processes. Active uptake may not be necessary because there is an electrochemical gradient of sodium ions across the cell membrane. Uptake of sodium and chloride ions into halophyte cells may also go through gated cation and anion channels, or even by vesicles. There is evidence indicating that ion transport from the apoplast to the vacuole in aboveground organs of salt-accumulating halophytes is carried out by means of pinocytosis.

To get the sodium ions into a vacuole they must be actively pumped into it because of the low concentration of sodium ions in the cytoplasm, while chloride ions might passively enter the vacuole via anion channels to balance the differences of electrical charge across the membrane. The uptake of sodium ions into the vacuole appears to be mediated by sodium/hydrogen antiporters in the tonoplast, working together with H+-ATPases and

perhaps PPiases that provide the proton motive force. The vacuolar Na+/H+ antiports are always active in some species while other species have the antiport active only with high sodium chloride concentrations. Some plants make more antiports to respond to increase of sodium chloride. Activating existing antiports or making new ones is a strategy, applied equally by salt tolerant glycophytes as well as halophytes. Only under conditions of increased salinity, salt-tolerant glycophytes tend to activate pre-existing tonoplast antiport molecules. In most halophytes species, even in plants grown in the absence of sodium chloride, vacuolar antiports are constantly activated. There is evidence to show that halophytes rapidly scavenge sodium ions from their environment and sequester it into the leaf cell vacuoles even at low external salt levels.[6]

The presence of chloride ions must have considerable significance in the process. In osmoregulation and salt-tolerance, chloride ions are just as important as sodium ions. Chloride ions are compartmentalized in the vacuole in very high concentration in salt tolerant plants. Fluctuations of chloride ion concentrations in the cytoplasm and vacuole have been shown to regulate the transport of other anions into the vacuole. The vacuoles have a uniport that allows chloride ions to accumulate in it in response to the membrane potential generated by H+ pumps. Accumulation of chloride ions can reach high amounts without the need of additional cellular energy.

Once the sodium ions are in the vacuole they are susceptible to leakage back into the cytoplasm because of the difference in concentration between compartments. That would mean that the sodium would need to be pumped back into the vacuole faster to compensate for the leakage. Leakage of sodium ions causing the vacuole to stop being positively charged with respect to the cytoplasm would also result in leakage of chloride ions. To prevent the sodium from leaking back some plants add highly saturated fatty acids and other lipid in their vacuole membranes to minimise the permeability of sodium chloride. Also, tonoplast cation channels are closed at physiological concentrations of sodium ions. If there is no leakage, a relatively small proportion of tonoplast H+ ATPase activity would be needed to maintain sodium chloride compartmentation. So energy wise, salt tolerance may not be expensive.[7]

Seeing that halophytes do take up and retain salt it is likely that they can specifically be used to remove salt from water and soil.

Use of halophytes as a means to solve the salt problem

In most use of halophytes as a method to reduce salt in the soil or use of wastewater for irrigation, the principle of leaching is important. Leaching allows part of the salts in the soil, to be removed with water that is not taken up by plants or evaporated. This is fine if the only concern is getting the soil ready for agricultural use, or getting the best yield with poor available water. But leaching of salts also means that the salt gets transported elsewhere. Thus, salt sensitive environments may get exposed. To prevent that, irrigation systems are used that supply less water than could be consumed in evapotranspiration of a well-watered crop. This assures that only a minimized discharge passes the root zone.

This is called deficit irrigation. While it minimizes the flow of salt into the environment, this type of irrigation method also results in reduced yield and a salinity in the root zone that is higher than optimal, so it can be used if needed for agriculture with poor water availability but not for soil reclamation. The basic idea of this type of irrigation method is that the plants will consume the incoming water before it can discharge past the root zone. This, then also prevents salts to be leached away and causes a build up of salt that makes this type of irrigation poorly usable for plants of low or moderate salt tolerance. The salt build-up within the root zone will eventually reach or exceed the salt tolerance limit of the plants, eventually killing them. When they die, no more water will be removed from the soil and discharge past the root zone will occur. Such an event will again result in the leaching of salt into the environment.
Therefore it may be advantageous to use halophytes when using deficit irrigation. Another problem with deficit irrigation is that the rate of effluent discharge might not be optimally matched to the seasonal water demand of the crop. While waste water produced from industrial or water treatment processes tends to be fairly constant over a year, the evapotranspiration of plants are highly seasonal. This could lead to discharge past the root zone during the winter because the evapotranspiration is lower while the water discharge remains at the same level. To avoid this problem plants with deep roots can be used because, to support the summer evapotranspiration, they can utilize the water that is stored in the soil in the winter. A good example of deficit irrigation can be found in the water disposal facility operating at Twentynine Palms, in the Mohave Desert, California. There, deeply rooted halophytes were used to absorb saline effluent from the local water treatment facility. Twentynine Palms, like many other desert communities, depends on groundwater for its drinking water, so all water projects need to minimize the discharge of salts to their aquifers, and all disposal schemes must be approved by the California Regional Water Board. The Twentynine Palms Water District had constructed a water treatment facility for the removal of fluoride in the production of drinking water. The wastewater that the facility produced, had elevated levels of sodium, chloride, sulphate and TDS that needed to be disposed of without major impact to the environment. A perennial halophyte shrub called *Atriplex lentiformis* (also known as big saltbush) was taken as a landscape plant to absorb the wastewater, using a deficit irrigation strategy and so minimizing the discharge past the root zone. *Atriplex lentiformis* was used because it is native to the Mojave Desert where it provides food and habitat for livestock and local wildlife. It can have a high productivity and can support high levels of evapotranspiration if water is available. It usually has a rooting depth of about three meters, but the roots can go as deep as five meters to reach the water table. It has been used before in other projects over the world for restoration of desert areas. It is a plant that produces substantial amounts of seeds. Those seeds germinate readily, so there is a steady source of replacement plants as a stand matures. An *Atriplex lentiformis* planting on six hectares of ground were established to absorb about 35,000 cubic meters of wastewater per year. *Atriplex lentiformis* produced nearly full plant cover on a deficit irrigation system. The low irrigation volumes resulted in a low leaf area compared to traditional crop plants, but comparable to those measured for natural stands rooted into a shallow, saline aquifers. This process using *Atriplex lentiformis* ended with an increase of sodium by about 247 to 380 milligrams per kilogram per year in the top soil but was successful at in preventing

discharge of water past the root zone and salinity did not become a constraint on other plant performance.[8]

Halophytes as product

As halophytes are able to live in saline environments, it is only natural that people consider their use when faced with the problems of fresh water shortage. If wastewater or seawater could be used to irrigate the fields, more areas such as deserts or sanddunes would be open for agricultural use. To accomplish that, either use must be found for halophytes as crops, or known glycophyte crops must be converted to halophytes.
So far there has been little success with getting acceptable crop yield out of conventional crops on high saline water in arid climate.
The other approach is the domestication of halophytes. There are halophytes that are already being used as a source of oilseed and straw that can be used for animal feed if balanced out with other food stuff. The protein contents vary over the different halophytes. Crop quality is also very much dependent on harvest time and the parts of the plants that are harvested. Using halophytes as crops does bring problems because they have not undergone the heavy selection the traditional crops have undergone over the centuries of agricultural history.[9] Another problem with halophytes as a crop, is that it by it very nature has a high salt content which can be problematic in the use as food.
For halophytes to be successful as crops, they need to have high yield potential, with easy irrigation requirements, comparable to conventional crops that do not damage the soil. The products must be able to fill the same need as conventional crop products. Also the high-salinity agriculture must have a role within the existing agricultural infrastructure. If the application of halophytes would require the creation of new infrastructures, it is unlikely that such a solution will be adopted. The oilseed yield potential of some halophytes is high, the yields being comparable to conventional forage crops such as alfalfa or Sudan grass in desert irrigation districts. Some crops have the potential to be used for human consumption, like the herbaceous plant *Atriplex triangularis*.[10]
A promising halophyte for crop use is *Salicornia bigelovii.* This leafless, succulent, annual salt-marsh plant has prolific seed production. The seeds have high oil levels and high levels of protein with salt content less than 3 percent. The oil is edible and can be made into biofuel and the seed meal can replace conventional seed meals at the levels normally used as a protein supplement in livestock diets.[11]

System for removing salt

The system that is currently needed, is a system to remove salt from the wastewater produced by the water cleansing facility at A.G. Wildervanckkanaal. The amount of wastewater produced is about 800.000.000 liters per year and the sodium concentration is about 6,35 mmol/l or 146 mg/l. This amounts to 5.080.000 mol or 116.800.000 grams of sodium per year

The wastewater will be irrigated into a basin with soil on which the halophytes will grow. The size of the basin and the amount of plants growing on it, depend on the type of plant that is used, because different halophytes take up different amounts of sodium. *Atriplex polycarpa* can take up 83,7 mg of sodium per gram of dry weight, while *Suaeda maritima* can have 126,5 mg of sodium per gram of dry weight. These levels are found when irrigated with water of 200 mmol/l sodium chloride, and lower levels are found at lower concentrations of sodium in the water. For instance *Atriplex polycarpa* has 55,7 mg of sodium per gram of dry weight if the irrigation water has a concentration of 100 mmol/l sodium chloride.[12 13] Considering the amount of 116.800 kilograms of sodium being produced per year, either 1.395.460 kilograms of *Atriplex polycarpa*, or 926.984 kilograms of *Suaeda maritima* would be needed each year. This being dry weight, the actual weight would be much larger. For *Atriplex polycarpa* as well as for *Suaeda maritima* growth problems, due to salt accumulation in the soil are not to be expected as the concentration of sodium in the wastewater of 6,35 mmol/l, is relatively low. The required amount of plant material for each species decreases with increase of sodium uptake of a plant. Growth rates for species with greater sodium uptake can be lower than for those with smaller uptake. To give a good description of the system, the pooled data of different dicotyledenous halophytes will be used of which the uptake and growth rate and fresh weight is known as is seen in fig 1.

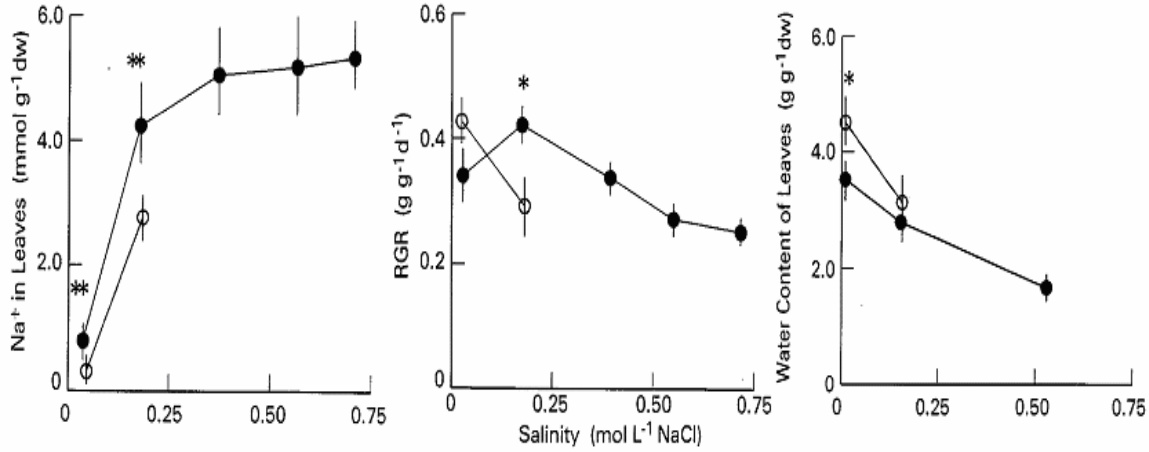

Fig 1. Growth of dicotyledenous halophytes along a salinity gradient in a greenhouse screening experiment. (open circles = less tolerant species, N = 10) (closed circles = more tolerant species N = 10). Panels show relative growth rates (RGR); Na+, water content of leaves. Error bars are SE of means across species.

The pooled dataset has for sodium content 4,1 mmol per gram dry weight, or 94,3 mg per gram dry weight and a relative growth rate in which it gains 0,41 grams a day for every gram. So for every gram it weighs, it gains an additional 0,41 grams a day. Thus, for every gram the plant weighs, it can take up an additional 38,663 mg of sodium each day. A total amount of 1.238.600 kilograms of dry weight is needed to take up 116.800 kilograms of sodium. Using the formula $W_n = W_1 e^{r(t_n - t_1)}$, it should be possible to

calculate the amount of plant material, needed at the start of the cycle, in order to end up with the right amount of plant material at the end.[14] If W_n would be taken for the 1.238.600 kilogram of dry weight, then r would be 0,41, t_n would be 365 and t1 would be 1. The result would be $W1 = 1238600 / e^{0,41*(365-1)} = 1,9003 *10^{-59}$.
This outcome seems rather small. Therefore, it may be preferable to consider accommodating the daily need. The daily amount of sodium that would be acquired with the wastewater is 319999996,8 mg and resulting in the need of 3393,425 kilograms of plant material per day. This leads to a starting weight of 2252,04735 kilograms. Within 17 days the level of available plant material would be reached, sufficient for the sodium uptake for the hole year ($t_n = (\ln(w_2/w_1)/r)+t_1$).
This formula may be helpful in predicting amounts needed or amounts to be gained, its accuracy, however, seems questionable. If, for example, the relative growth rate is 0,41 grams per gram a day and the starting point is indeed at 1 gram, it is to be expected that the next day there would be that gram plus the relative growth rate, being 1,41 gram. The formula, however, would give $W_2 = 1*e^{0,41*(2-1)} = 1,507$ gram, suggesting an overestimation of what is actually gained. These calculations were done with the assumption that the sodium uptake and relative growth rate is the same at concentrations of 200 mmol/l as with concentrations of 6,35 mmol/l. This, most likely, is not accurate, as there are no data for the sodium uptake and relative growth rate at concentrations of 6,35 mmol/l. Also, it is unlikely that the growth rate is constant all year. No accounts for the effects of seasonal fluctuations on sodium uptake could be made.
The size of the basin may not be smaller than the amount of wastewater produced per day so the basin must be at least 2191,7808 cubic meter. Of course the basin has to hold more than just the water. It needs to accommodate the soil as well, and it has to be taken into account that there is a distinct possibility that not al the waste water will be absorbed, consumed, and processed each day. The amount of water that the soil can absorb is important for the volume of the basin because the amount of water absorbed does not occupy additional volumes of space. The basin must also be able to have enough space to carry 3393,425 kilograms dry weight of plant, which is estimated to be about 10180,275 kilograms of fresh weight. The basin needs to be deep enough for the roots to grow properly. In the absence of specific data on root depths, the assumption is made that the depth dose need not to be deeper than three meters.
The volume of the basin also depends on the water uptake and evapotranspiration of the plant. Another point of consideration is the surface area needed for each individual plant. *Atriplex polycarpa* for instance, can grow as high as 200cm (widths 100-200cm), whereas *Suaeda maritima* generally reaches only a height of some 30cm. Therefore it will take larger surface areas for the latter to reach the required amounts of dry material. To truly make a good system one must, aside from the sodium uptake of the plant and relative growth rate, study the evapotranspiration, root depths, preferred soil type, and shape of the plant one wants to use. Also, excess of plant should be removed, so a little bit of gardening is in order.
Considerable attention needs to be given to the type of soil on which the halophytes are grown. Soil type is very important in not only how fast water is absorbed into soil, but also in water retention (how much is held in the soil as opposed to how fast it drains out). Some soils take up water very slowly, such as clay soils, and also hold it well (they do not drain well). Some soils absorb water well, such as peat and sandy soils. Peat holds

the water well (doesn't drain very fast because of the high organic content) whereas sandy soils do not hold water well, allowing it to drain out fairly quickly[15]. The soil used must be tailored to the preferences of the plant that is being used. The wastewater already contains a lot of nutrients so none, or little, fertilisation has to take place. Surrounding the basin must be a layer that is impenetrable to water, like clay. This would prevent leakage of water, and leaching of sodium salts, into the outside environment.
To spread the water evenly over the basin, an irrigation system is needed. The most appropriate system that could be used, seems to be one where evenly divided channels are made in the soil with a downward slope. These channels run parallel with one another, so that water released into these channels will spread evenly over the entire area by gravity. This type of irrigation is know as furrow irrigation.

Summary

The use of halophytes for waste water processing and for agricultural purposes is discussed The mechanisms to take up and distribute sodium in halophytes are found to be useful in preventing against saline pollution of the environment. An economically valid and reliable system of removing salts from wastewater appears plausible.
The use of halophytes is recommended for the desalination of waste water at an industrial site near A.G. Wildervanckkanaal. Some of the requirements on volume, lateral space, soil types for such a facility are addressed.

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