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Scienze Agrarie

Curriculum Scienze e tecnologie zootecniche.

Ciclo XXIX

MECHATRONICS APPLICATIONS AND PROTOTYPING SENSORS FOR THE PRECISION LIVESTOCK FARMING

Dr. Giovanni Chessa

Coordinatore del Corso Prof. A. Cannas

Referente di Curriculum Prof. G. Battacone

Docente Guida Prof.A. Pazzona

Anno Accademico 2015-2016

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RVRALIS ET SILVESTRIS SAPIENTIA

Curriculum Scienze e tecnologie zootecniche.

Ciclo XXIX

Anno Accademico 2015-2016

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Ciclo XXIX

La presente tesi è stata prodotta durante la frequenza del corso di dottorato in Scienze Agrarie dell'Università degli Studi di Sassari, a.a. 2015/2016 - XXIX ciclo, con il sostegno di una borsa di studio cofinanziata con le risorse del P.O.R. SARDEGNA F.S.E. 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1 "Finanziamento di corsi di dottorato finalizzati alla formazione di capitale umano altamente specializzato, in particolare per i settori dell'ICT, delle nanotecnologie e delle biotecnologie, dell'energia e dello sviluppo sostenibile, dell'agroalimentare e dei materiali tradizionali".

La tesi è stata prodotta, altresì, grazie al contributo della Fondazione di Sardegna.

Giovanni Chessa presents its sincere thanks to the Sardinian Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007-2013 - Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.).
Sincere thanks also to the Fondazione di Sardegna for the financial contribution.

Sommario

INTRODUCTION

The challenges facing agricultural and livestock sciences are summarized in a FAO report with the deliberately explanatory title ‘Can we feed the world in 2050?' (FAOSTAT, 2009). The projections in this report indicate that by 2050 the world population will be over 9 billion - currently we are just over 7 billion - of which over 70% will settle in urbanized areas - compared to 49% in 2009 - and the greatest contribution to global population growth will be given by developing countries. A later report, ‘World Livestock’ (FAOSTAT, 2011), estimated an increase in meat consumption of 73% by 2050, while dairy products consumption will rise by about 58%. To meet such a demand for animal proteins in line with the present state of efficiency of the livestock sector, the increase in breeding of poultry should be by 100%, that of small ruminants by 80%, cattle farming by 50% and pig rearing by 40%, without changing the current level of exploitation of natural resources. Several authors (Avery, 2001; Reilly and Willenbockel, 2010; Pulina et al., 2011) have drawn attention to the constant reduction in the available and edible area per capita as a result of population growth and, to a greater extent, of the different land uses (nature reserves, urban, energy production). In a globalized world food security must also be addressed under the qualitative aspect and the Directorate General for the health of EU consumers, being well aware of this, expressed deep concern in the EU 2011 report about the diseases that may be transmitted by animals to humans and the excessive use of antibiotics in livestock farms.

The average consumer is increasingly concerned with the quality and origin of food products and if in the past products from systems guaranteeing high animal welfare levels did not exceed 10% of the market share (Webster, 1999), today this percentage is on the increase. Significant to that effect is the result of a study (Furnols et al., 2011) indicating how consumers would not purchase lamb meat if of dubious origin. Terms such as aflatoxin, dioxin and Escherichia coli are beginning to be part of the average consumer language, resulting in a more careful and refined purchase.

In line with the current scientific knowledge, and given the great diversification in technological and managerial progress of animal farms, there is still significant scope for increasing the efficiency of the whole agricultural and livestock sector. Scientific research has employed various types of sensors and data transmission technologies in order to increase the efficiency of the animal farms. The miniaturization of these technologies and the reduction of their manufacturing costs enable real-time monitoring of the environmental and biological parameters of the livestock sector. More specifically, the sensors that can measure both environmental parameters (dust, CO, CO2, NO2, O3, CH4, H2S, NH3, temperature and radiation), and biological ones (such as breathing rate, body temperature, animal position, muscle and heart activity) are nowadays processed by a single microcontroller and available at affordable rates.

Precision livestock farming (Plf) is the discipline that encompasses this approach of farm management (Berckmans, 2014) and allows to monitor in real-time the numerous biological and environmental parameters concerning each individual animal of the herd. A Plf system is always made up by three components: a physical element, i.e. the hardware; an element for data processing and presentation, known as the software; and an element for the transmission of data, i.e. the network. The hardware comprises the sensors, the computers and/or microcontrollers, the data transmission and acquisition systems and the actuators. Mathematical models for data processing and the data presentation interface are included in the software loaded into the microcontroller. Table 1 shows the wide range of application of Plf and their possible monitoring frequency (Durack, 2002; Berckmans, 2014).

Table 1. Summary of the possible environmental and operational variables that can be measured, recorded and analyzed in Plf (Durack, 2002 and Berckmans, 2014).

Plf application areas	**Sampling frequency**
Biological parameters	
Daily weight gain (IPG)	Daily
Food conversion index (ICA)	Daily
Feed consumption (CM)	Hourly
Body mass index	Daily
Normal/nervous behavior	Daily
Estrus activity detection	Hourly
Environmental conditions	
Outdoor and indoor climatic conditions (sheds, rooms)	Hourly
Floor temperature and humidity	Hourly
Air speed and ventilation rate	Hourly
Gas levels, such as CO_2 and NH_3	Hourly
Dust levels	Hourly
Traceability and transport	
Electronic product code	-
Environmental conditions during transport	Hourly
Animal feed and medicines unique codes	-

Several authors have contributed to the development of the fields of application of Plf, such as advanced systems for controlling environmental parameters (Banhazi et al., 2008), sensors for the assessment of animal welfare and behavior (Shao and Xin, 2008), systems for real-time appraisal of animal body weight (Kollis et al., 2007), real-time monitoring of water and feed consumption (Madsen and Kristensen, 2005; Madsen et al., 2005), employment of sensors in the diagnosis and monitoring of diseases (Maatje et al., 1997; Eradus and Jansen, 1999), electronic identification (Naas, 2001; 2002), recording and interpretation of animal verses (Holst, 1999). The incessant technological development constantly offers potential improvements and opportunities to implemented systems, even to the most advanced ones.

During the three years of my doctorate study I have sought to develop different issues concerning Plf, from the point of view of animal welfare as well as the application of new technologies in order to optimize business performance.

The study, subdivided into 5 chapters, comprises a review of the advancements of the main components of Plf, the development of a prototype for EC monitoring in ewe milk, a prototype for monitoring animals body temperature, the optimization of collection rounds of goat milk and the development of a prototype for somatic cell count (SCC) through the measurement of Sodium ions in ewe milk.

The first chapter is a review of the advancements of the main components of Plf, i.e. software, hardware and data transmission, focusing on issues related to hardware modularity and differences between licensed and unlicensed software. From the review it emerges that image processing is one of the most used techniques in Plf systems, in that it allows the detection of behavioral, biological and pathological parameters without interfering with the animals routine activities. In this regard the area occupied by a lamb carcass was calculated by using an image analysis open source software, CellProfiler (Jones et al., 2008).

The second chapter deals with the realization of an innovative portable tool for somatic cells count in ewe milk by measuring its electrical conductivity. There are over 15,000 dairy sheep farms in Sardinia, which represent both historically and economically the most important agricultural and livestock sector in the island. Indeed, Sardinia holds more than 40% of the national sheep population thanks to more than 3 million sheep heads that provide about 60% of the total national milk production. One of the most common problems in sheep farms is mastitis, an intramammary infection which may cause a quantitative reduction up to 50% in milk production and a qualitative drop, in particular of lactose and casein. One of the indirect methods for the assessment of somatic cell count (SCC) in ruminants' milk is through the measurement of its electrical conductivity (EC). In small ruminants, EC has a reasonable correlation R2 = 0.35 with somatic cells but to date there is still not a portable tool that can estimate SCC based on the milk's EC reading. The prototype was calibrated on Sarda ewe milk.

The aim of Chapter 3 was to develop a system using a open source sensors, actuators and micro-controller. The system is able to monitoring the rectal temperature of the animals, sending data via Bluetooth to a smart phone. The micro-controller used was an ATmega32U4, the temperature was read using the LM35 analogic sensor and a Class 1 Bluetooth serial module was connected to Arduino creating a wireless serial link between an Android phone and the Arduino board. The application for receiving data on an android smart phone was created using App Inventor that is an innovative Android application creation software developed by Massachusetts Institute of Technology (MIT). This app is free available on Google Play Store under the name *animal_temp*.

The costs of sheep milk collection rounds in Sardinia have been analysed in chapter fourth. The escalating costs incurred by the dairy processing industries for milk collection from individual farms have focused the attention on the rationalization of milk collection and transport systems. In this regard, the case of the Sardinian goat sector has characteristics that make it unique and not comparable to other logistics optimization realities. The problems of this sector are mainly represented by the particular conditions of the rural road network and the fragmented nature of livestock farms.

The aim of the present study was to test a milk collection route optimization software, MilkTour, in the collection rounds of a sample cheese dairy. The software has been developed by the Land Engineering Section of the Agriculture Department of the University of Sassari. A total of 5 routes were analysed and optimized.

The results have highlighted the importance of optimizing collection routes as they have a significant impact on business costs. A important contribution that has emerged is the strong correlation between collection density and the cost per litre of collected milk (€cent/l), which allows to detects the cost-effectiveness of a round of collection and its relative optimized around.

The objective of chapter 5 was to study the relationship between the ione Na^+ and the main components of sheep milk, in particular somatic cells. Moreover, a portable device for estimating SCC in sheep milk was designed. The study was conducted on over 2000 samples.

The milk components examined were: fat, proteins, lactose, pH, sodium chloride, urea and the ions Na^+. The correlation between Na + and SCC corresponded to $R^2 = 0.76$ (P <0.01).

The prototype developed incorporates two containers which receives milk samples taken from each half udder. Each container has integrated inside two sensors, one to detect the level of Na^+ in the milk and the other one to compensate the milk temperature. The mathematical model, loaded into the microcontroller by a firmware written in C / C ++, analyze the data and gives back the estimate of SCC level, so it allows farmers to monitor the ewes health status by periodically comparing the somatic cell counts of each half udder.

1. PLF REVIEW

1.1 Hardware review

1.1.1 Sensors

Plf systems sensors can be subdivided into two broad categories: those designed to detect environmental variables and those to detect biological variables. Typical environmental parameters are: temperature, humidity, air speed, air particulate matter, CO_2 and NH_3 concentrations, odour levels, and water quality. The main biological parameters are: body temperature, estrus activity, heart rate, breathing, rumination, brain activity, motor activity, infections, lameness and feeding. Plf represents a typical automatic control system with an input and an output. For instance, in a system designated to detect estrus activity a pedometer might be useful so that by monitoring an increase in the bovine motor activity, it signals the event to the farmer. This is a simple system to be implemented both at a hardware and software level, however in reality, of course, there can be a bovine suffering from lameness that is also in an optimal condition for insemination. In this case, the pedometer records a reduced or absent motor activity but is unable to detect estrus activity. Therefore, a system capable of monitoring all these variables by interpreting any deviation from expected values needs to be complex both at a hardware and software level. In order to achieve a precise control it is essential to elaborate a mathematical model that encompasses the highest possible number of representative variables of the monitored animal. The main features that a sensor to be used in Plf systems must have are: accuracy, reliability, sturdiness and the capacity to record and transmit data. The accuracy of a device, expressed in terms of the deviation from the expected value, must not exceed 5%. On the market there are more expensive devices capable of transducing the same parameters but with an accuracy of less than 1%, consequently the choice of the accuracy percentage should be dictated by the type of parameter to be monitored. As for reliability and sturdiness, it is worth bearing in mind that some commercially available sensors are not suitable for usage in livestock farms and require, therefore, a hardware upgrade to be, for example, water, mud and dust proof. Moreover, it may often be necessary to upgrade the sensors' software in order to enable data recording.

1.1.2 Microcontroller

Microcontrollers constitute a system that integrates on the same chip a processor, permanent and volatile memory and input-output channels (I/O), as well as any other specialized peripheral (Figure 1). These devices have the ability to receive incoming data from sensors and treat them first with any appropriate filtering blocks and analog-to-digital conversion. Microcontrollers are then able to activate actuators after processing the data with a specific algorithm.

A typical automatic control system is composed of: a transducer, which acquires the system's parameter to be controlled; an analog-digital converter (A/D); a microcontroller, which processes the transduced parameter and sends the appropriate control signal to the actuator (Figure 2).

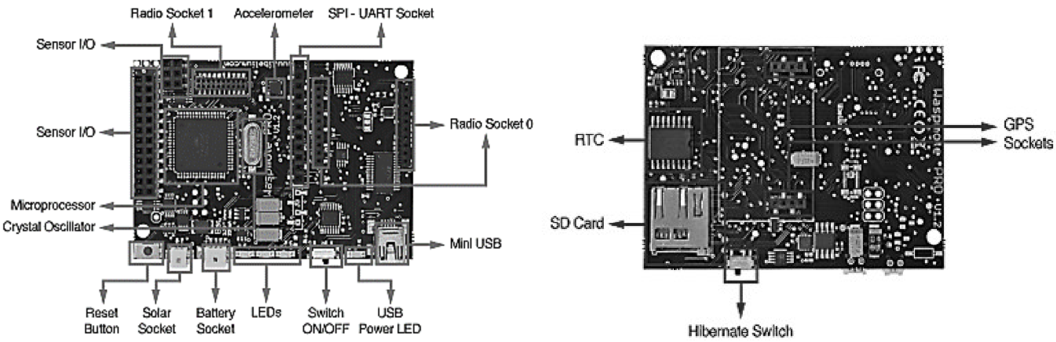


Figure 1. Left: top view of the Waspmote board microcontroller, and highlighted in red, starting from the bottom clockwise: button for card reset, microprocessor, pins for the insertion of sensors, accelerometer, pins for connection to the data transmission modules, mini-USB port for connection to a PC and to a power supply and finally the socket for connection to battery or photovoltaic panel. Right: bottom view, highlighted in red: slot for the SD memory card and the pins for GPS insertion.

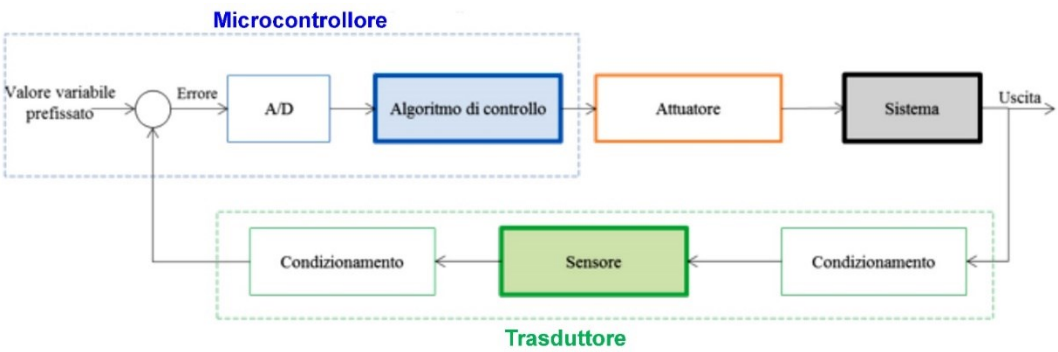


Figure 2. Block diagram of a typical automatic control system. In blue the microcontroller that performs the function of data acquisition from the transducers, marked in green; it then processes them through the control algorithm and finally sends the appropriate commands to the actuator to minimize the error between the desired and the measured value of the controlled variable.

A simple example of an automatic control system is the regulation of temperature and humidity inside the barn / pen in response to animals' body temperature (Figure 3).

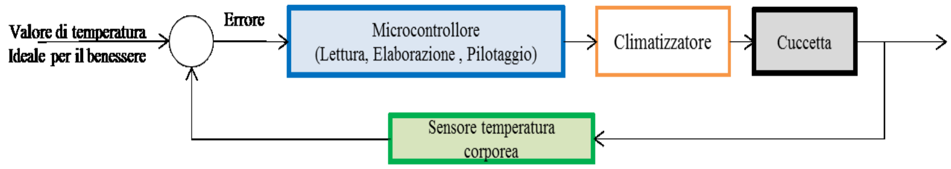


Figure 3. Block diagram of an automatic control system for animal thermal comfort in a pen. This is a typical example of a single-input and single-output system (SISO).

As the animal's body temperature changes, the microcontroller activates the cooling or heating system of the pen. This is a typical example of a single-input and single-output system (SISO). There are three other systems: SIMO (Single Input Multiple Output); MISO (Multiple Input Single Output); MIMO (Multiple Input Multiple Output). The Plf real potential can only be optimized by using a MIMO system (Figure 4).

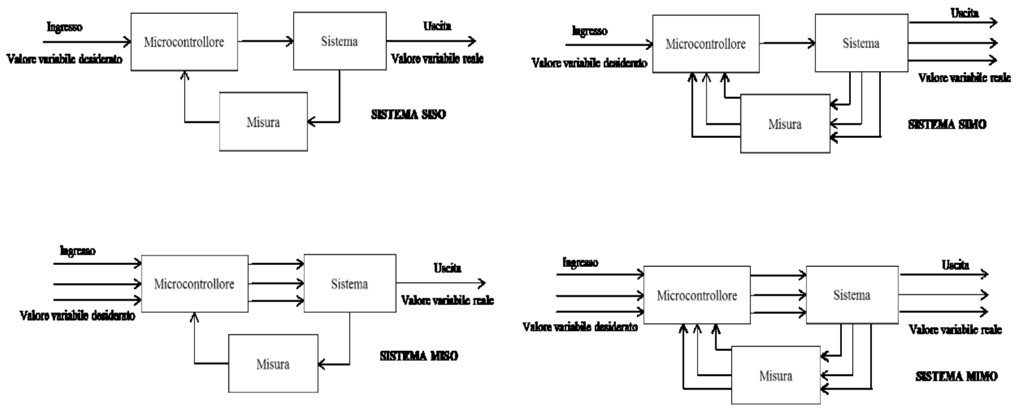


Figure 4. Schematic representation of SISO systems (single input and single output), SIMO (single input and multiple outputs), MISO (multiple input and single output) and MIMO (multiple inputs and multiple outputs).

For instance, it may not always be possible to control an increase in a housed animal body temperature by cooling its pen due to several factors that may came into play such as the immune system's response (infections or wounds), heat or other external events. In this case the system must be able to handle more parameters at the same time in order to identify the real cause of the body temperature increase and perform the correct control action.

1.2 Software review

1.2.1 Commercial VS open-source software

Plf is an embryonic technology with great potential, although it still requires intensive research and development before it is perfected and can be used to full capacity (Wathes et al., 2008). The lack of hardware modularity and relative closed source software (commercial or licensed software) of automatic systems offered by manufacturers are the main factors that hinder Plf development. Many of the systems available on the market today, in fact, do not allow to add additional sensors to the system, if not those by the same trademark, and often manufacturers do not offer a complete range of sensors for all transducible parameters within the farm (Table 2). The aim of research must therefore be to create a free, modular platform open to the evolution of new technologies. Scientific research has already introduced in other fields modular and free (open-source) systems that have proved to be very successful: for instance VEGA (Pedretti et al., 2004), an open platform for the development of bioinformatic applications with plug-in architecture and script programming; NA-MIC (Pieper et al., 2006), an open platform of medical imaging for the computing community; the humanoid robot iCub (Metta et al., 2008), an open platform for research on cognitive processes.

Table 2. Comparison of sensors available on different Plf systems for pig farms (Banhazi et al., 2003). Skov and Farmex represent the most equipped systems for transducible parameters although no system in the table below is able to measure the level of odour inside and outside the pigsty as well.

	Temperatura stalla	Umidità stalla	Velocità del vento stalla	Direzione del vento stalla	Temperatura esterna	Umidità esterna	Velocità del vento esterna	Direzione del vento esterna	Livello polveri stalla	Livello CO_2 stalla	Livello NH_3 stalla	Livello odore stalla	Livello odore esterno	Mangime consumata	Acqua consumata	Peso maiale	Livello sudorazione	Registrazione audio	Registrazione video	Livello ingrassamento (back fat)	Calore	••••••••••••••••••••••	Feedback del mercato	Supporto	Controllo automatico	Accesso privato	Rete	Ingresso dati manualmente	••••••••••••••••••••••	Caricamento nel Pc	Software di analisi	Importa	Esporta	Conserva	Protocollo
Farmex	√	√	√	√	√	√	√	√	•	•	•			√	√	•	•	•	•	•	•	*	•	√	√	√	√		*	√	√	√	√	web	
Environdata	√	√	√	√	√	√	√	√														*		√		√			*	√	√			pdb	rs232, modem
Ruddweigh																√						*		√		√			*	√				csv	rs 232
Skov	√	√	•	•	√	√	•	•	•	•	•			√	√	√				•	•	*	•	√	√	√	√		*	√	√	√	√	pbd	
Piggery Systems & design	√				√																	*		√	√	√			*	√					
Hotraco (B&M Slots)	√	√		√	√	√		√		√	√			√	√							*		√	√	√	√		*	√	√		√	asc	rs232/modem
Big Dutchman	√	√		√	√									√	√	√						*		√	√	√	√		*	√					
Veng System	√	√	√	√	√	√	√	√	√	√	√			√	√							*			√	√	√		*	√			√		rs232
Multifan	√	√			√	√																*			√		√		*	√					
Microfan	√	√			√																	*			√	√			*	√					
Rotem	√	√		√	√									√	√							*			√	√			*	√					cable, modem
Fancom	√	√			√	√				√	√											*			√	√			*	√	√				cable, modem
Watchport	√	√			√	√																*				√	√		*	√					usb/ ethernet
Phason	√				√									√	√							*			√	√	√		*	√	√				
bsmagri	√																					*			√				*						
chretronics	√	√																				*			√	√			*	√	√				cable, modem
Ozonaire												√	√									*			√				*						
Biocurtain												√	√									*							*						
VIA - (osborne)																√			√			*				√			*	√	√				cable
Ethovision																			√			*				√			*	√	√				cable
Farmweld																√						*			√				*	√	√				wireless
Osborne	√	√												√		√					√	*			√	√			*	√	√	√	√	csv	rs232
tru-test scales																√						*							*	√				csv	rs232 + rs485
skiold/ sorti-pen																√						*			√				*	√					
IVOG														√		√					√	*							*						
Mannebeck														√						√	√	*		√		√		√	*	√					
Sono-grader																				√		*							*	√	√	√	√	csv	handheld upload
Lean meater																				√		*							*						
Pigtog 105																				√		*							*	√					
Super tester																				√		*							*						

1.2.2 Mathematical model

A key point in Plf is the selection of a suitable algorithm capable to represent, as closely as possible, the reality of the phenomenon observed/detected by the sensors. Mathematical modeling is crucial in the assessment or classification of a variable or an event. The results of two systems for the prediction of cattle estrus cycle are compared in Table 3: the HR Tag (SCR Engineers, Ltd., Israel), which monitors head movement and rumination, and IceQube sensor (IceRobotics, Ltd., Scotland) which monitors physical activity. Specificity indicates the ratio/probability that a cow is negative to the test (no estrus), while sensitivity indicates the probability that a cow on heat proves to be positive to the test (estrus). The best results are achieved with neural networks, i.e. machine learning techniques that reach 100% sensitivity with a combination of both technologies. Self-learning or supervised learning algorithms have recently been developed for regression and classification, such as support vector machines and neural networks. Self-learning models, through which a sort of virtual memory and cataloguing experience, comparable to the farmer's, is created, can become all the more precise and efficient as the available data increase. These models, which require a huge amount of data, are implemented on increasingly miniaturized and powerful computers.

Table 3. Comparison of classification accuracy of several mathematical models using the same sensor (Borchers, 2015).

Mathematical model	Technology	Sensitivity[1]	Specificity[2]
Random forest	HR Tag	44,4%	95,3%
	IceCube	88,9%	98,2%
	HR Tag + IceCube	88,9%	98,2%
Linear discriminant analysis	HR Tag	77,8%	88,8%
	IceCube	77,8%	98,2%
	HR Tag + IceCube	77,8%	97,6%
Neural network	HR Tag	55,6%	91,8%
	IceCube	88,9%	93,5%
	HR Tag + IceCube	100,0%	96,5%

1 Sensitivity = True positives / (True positives + False negatives) x 100

2Specificity = True negatives / (True negatives + False positives) x 100

1.3 Plf sensors review

The farmer monitors his herd through sensory analysis and is aided in the decision-making process by his experience gained over the years (Wathes et al., 2008), however the increase in the number of stock units per farm has made direct monitoring increasingly challenging (Guarino, 2005). Plf allows the implementation of automatic systems able to monitor, inter alia, the animals physical activity (Cangar et al., 2008, Aydin et al., 2010), traceability (Sergeant et al., 1998 ; Kashiha et al., 2013), behaviour (Leroy et al., 2006), welfare (Song et al., 2008; Poursaberiet al., 2010; Viazzi et al., 2011) and growth rate (De Wet et al., 2003; Demmers et al., 2012). One of the main purposes of Plf is to provide a support to the farmer in the observation of his livestock and individual animal, using sensors and monitoring techniques such as, for example, image and sound analysis. These techniques allow to obtain the necessary information without subjecting the animal to invasive detection methods which, by subjecting the animal to stress, may distort measurements (Cangar et al., 2008).

1.4 Sound analysis

Farm animals vocalization analysis is a non-invasive method that allows the evaluation of different parameters such as their welfare and behaviour (Watts and Stookey, 2000; Schön et al., 2003). This system allows the monitoring of behavioural, pathological and production parameters such as, for example, the quantity of dry matter ingested by livestock (Schirmann et al., 2009), their behaviour (Deshmukh et al., 2012), the identification and localization of stock units (Klindtworth et al., 1999; Rossing, 1999; Ikeda et al., 2003; Marx et al., 2013), time spent chewing (Laca and Wallis De Vries, 2000), estrus activity (Kim et al., 2010), lameness (Whay et al., 1998; Rajkondawar et al., 2002) and emission levels of metabolic gases causing air pollution (Van Hirtumand Berckmans, 2002). Studies conducted so far have focused mainly on housed pig (Weary et al., 1998), poultry and cattle farms (Schön et al., 2007) albeit only to a certain extent on the equine (Moehlman, 1998), sheep (Walser et al., 1981; Kendrick et al., 1995) and goat sectors (Shelton, 1980).

1.5 Image analysis in livestock farm

Image analysis is a non-invasive technique to acquire behavioural, productive and health information in different species. Some studies have used image analysis techniques to evaluate, for example, thermal comfort that significantly affect milk production and the behaviour of cattle (Kadzere et al., 2002; Bohmanova et al., 2007), pigs (Shao and Xin et al. 1998 Xin and Shao et al. 2008) and broiler chicks (Cassuce et al., 2013), while there are no studies available on semi-housed and free range livestock. Through the evaluation of specific stock anatomical areas it is possible to calculate the live weight or fattening state - Body Condition Score (BCS) - in pigs (Brandl and Jorgensen, 1996), dairy and beef cattle (Schofield, 1990; 1992), buffaloes (Negretti et al., 2008) and chickens (De Wet et al., 2003), while there are no studies available on horse, sheep and goats farms, for which the hand method is used (Santucci and Maestrini, 1985; Thompson et al., 1994).

Monitoring the animals BCS and weight represents an important indicator in farm management: a very fat heifer, for example, may run into difficult calving, while its excessive thinness may cause a decrease in milk production and quality. Recently, thanks to more efficient algorithms and software, it has been possible to introduce extremely accurate weight measurement systems for pigs and cattle (Wang et al., 2008, Kashih et al., 2014; Ozkaya et al., 2015). Recently, thanks to more efficient algorithms and softwares, it has been possible to introduce extremely accurate weight measurement systems for pigs and cattle (Wang et al., 2008, Kashih et al., 2014; Ozkaya et al., 2015). Image analysis techniques have also been used to monitor behaviour and to evaluate states of panic or discomfort in pigs (Costa et al., 2014), cattle (DeVries et al., 2004; Stubsjøen et al., 2009) and chickens (Porto et al., 2013). Furthermore, with image analysis it is possible to monitor some pathological conditions such as lameness in horses (White et al., 2008) and cattle (Van Hertem et al., 2015). Of equal significance is the possibility to check feeding times and feed consumption in poultry farms (Mehdizadeh et al., 2015) and dairy cattle (Porto et al., 2012; Shelley and Anthony, 2013).

There are many types of licensed software for image analysis providing different functionality related to the product price, whereas open source ones are: ImageJ, Fiji , OpenCV (the most feature packed one though with no graphical user interface), Simplecv and CellProfiler . The latter was created for biologists with no computer programming training to analyse cells images. Il software è dotato di una serie di moduli che consentono si rilevare diverse grandezze dall'analisi dell'immagine, ma una delle potenzialità più interessanti del software è la possibilità di modificare moduli o di crearne di nuovi per soddisfare particolari esigenze (Carpenter et al., 2006; Lamprecht et al., 2007; Jones et al.,2008; Stöter et al., 2013; Bray e Carpenter, 2015). By way of example, the image analysis of a lamb carcass was used after it had been selected using a Google-specific search engine. Before proceeding to the evaluation of the area occupied by the carcass it was necessary to convert the image from RGB to grey scale (Figure 5), an option available in the image processing modules of the CellProfiler software. The software allows the selection of modules that enable the extraction of different parameters such as the area, the intensity, the perimeter and the granularity (Figure 6).

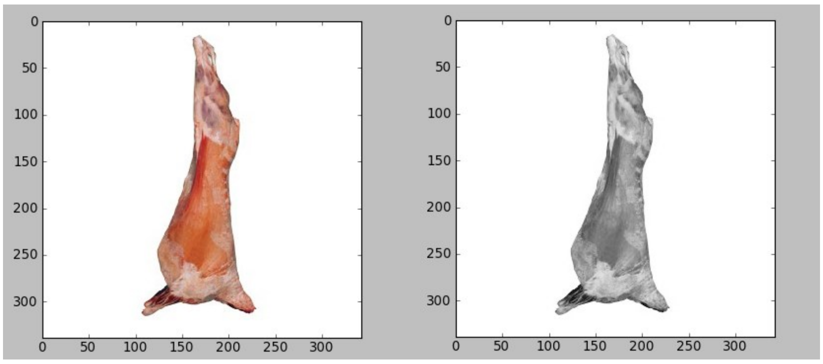


Figure 5. Image conversion prior to its processing: on the left the original image, on the right the lamb carcass after conversion from RGB to gray scale.

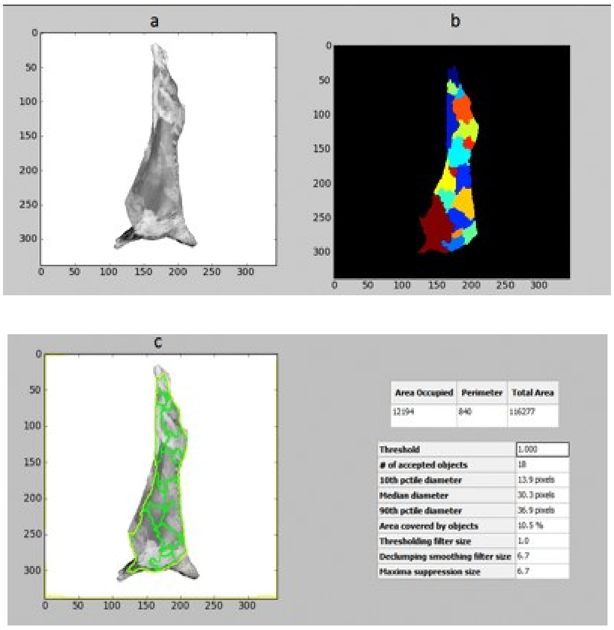


Figure 6. Example of calculation of the area occupied by the image of the lamb carcass through CellProfiler software..

1.6 Hardware modularity & commercial software problems

The lack of an open source software coupled with the low hardware modularity represents a hinder to an increased use of Plf. Different platforms for rapid prototyping are currently available on the market, including: Arduino (Interaction Design Institute Ivrea, Italy), Waspmote (Libellium CTO, Calle Escatrón, 16, 50014 Zaragoza, Spain), Raspberry Pi (PiRaspberry Pi Foundation, Caldecote, South Cambridgeshire, UK). These platforms are modular, low cost and with a large active web community, ranging from engineering to agricultural scope of practice. For instance, the Waspmote microcontroller, characterized by high speed and low power consumption, has an integrated temperature sensor that allows a reading from -40ºC to +85ºC with an accuracy of 0,25ºC (Table 4). The card is also fitted with an accelerometer ±2g/±4g/±8g and can also be fitted with a JN3-Telit GPS model, which allows to obtain information such as latitude, longitude, altitude, speed, direction, date and hour.

Table 4. Waspmote microcontroller technical specifications: being lightweight with low power consumption it is ideal for application in Plf systems.

Microcontrollor	**ATmega1281**
Frequency	14MHz
SRAM	8KB
EEPROM	4KB
FLASH	128KB
SD card	2GB
Weight	20gr
Dimensions	73.5 x 51 x 13 mm
Clock	RTC (32KHz)
Consumption on	15mA
Consumption sleep	55uA
Consumption hibernate	0.7uA

The most interesting feature of the Waspmote microcontroller is the possibility of connecting additional cards for monitoring various environmental and biological parameters (Figure 7). The intuitive connection of the cards to the microcontroller requires minimal technical knowledge, although programming the cards may take some time. Thanks to the many additional components available it is possible to measure various environmental, chemical, biological, physical and mechanical parameters. Its range of application include: air pollution and air quality monitoring, farms emissions, environmental values in greenhouses, industrial processes emissions, forest fires, drinking water quality and pollution levels in rivers and seas monitoring, goods tracking, animal traceability, vibration and pressure detection, liquid and solid materials levels control, meteorological parameters check, fruit diameter, leaf wetness and soil moisture values detection (Table 5).

Figure 7. The electronic card for Gas monitoring is already wired and needs to be inserted only in the appropriate Waspmote pins; furthermore the sensors are already calibrated and are supplied with a certification, key parameters for distinguishing between a sensor for hobby measurements and one for scientific ones.

In addition, the card for quick prototyping is fundamental, as it allows the creation and integration within the Waspmote microcontroller of any sensor: this card represents the solution to the problem of hardware modularity, one of the critical points of Plf systems, that is, the possibility of connecting new sensors available thanks to future innovations without disrupting the system already implemented in the farm.

Table 5. Chemical, physical, mechanical and environmental parameters that can be measured with the various sensor sockets configured on the Waspmote controller.

Parametri Chimici / Fisici				**Meccanici**	**Ambientali**	
Carbon Monoxide	CO	Calcium Ion	$Ca2^{+}$	Pressure	Air temperature / Humidity	
Carbon Dioxide	CO_2	Fluoride Ion	F^{-}	Inclination	Soil Temperature / Moisture	
Molecular Oxygen	O_2	Fluoroborate Ion	$BF4^{-}$	Vibration	Leaf Wetness	
Ozone	O_3	Nitrate Ion	NO_3^{-}	Impact	Anemometer	Ozone
Nitric oxide	NO	Bromide Ion	Br^{-}	Hall Effect	Solar Radiation	
Nitrogen Dioxide	NO_2	Chloride Ion	Cl^{-}	Luminosity	Ultraviolet Radiation - UV	
Sulfur Dioxide	SO_2	Cupric ion	Cu^{2+}	Temperature	Trunk diameter	
Ammonia	NH_3	Iodide Ion	I^{-}	Liquid Presence	Stem diameter	
Methane	CH_4			Liquid Leakage	Fruit diameter	
Molecular Hydrogen	H_2	pH		Liquid Level	Wind Vane	Molecular Hydrogen
Hydrogen Sulfide	H_2S	Dissolved Oxygen		Weight	Luminosity	
Hydrogen Chloride	HCl	Conductivity		Noise Level	Ultrasound (distance measurement)	
Hydrogen Cyanide	HCN	Temperature			Oxidation-Reduction Potential	
Phosphine	PH_3	Turbidity			Atmospheric Pressure	

Data transmission represents one of the most delicate points of the Plf systems. The Waspmote microcontroller offers extreme flexibility in the transmission and reception of data through a variety of communication protocols, such as: 802.15.4 / ZigBee, LoRa 868 / 915MHz, Bluetooth Low Energy (BLE) 4.0 and Wifi (Table 6). There are also communication systems available on the market that are capable of transmitting over large distances such as the XBee-900 module, that can receive and transmit data over a range of 10 km. Furthermore, it is possible to encrypt communication, if the need arises, with the AES 128b key.

Table 6. Overview of the different communication protocols of Xbee type available for the Waspmote microcontroller. An increase in data transmission distances correspond necessarily a greater energy consumption in communication modules. Power consumption is a parameter to be monitored during the design of Plf systems

Model	**Protocol**	**Frequency**	**Tx power**
XBee-802.15.4	802.15.4	2.4GHz	100mW
XBee-ZB-Pro	Zigbee	2.4GHz	50mW
XBee-868	RF	868MHz	315mW
XBee-900	RF	900MHz	50mW

2. MILK ELECTRICAL CONDUCTIVITY IN SARDA BREED SHEEP: A PROTOTYPE OF A PORTABLE DEVICE FOR ESTIMATING THE SOMATIC CELL COUNT

2.1 Introduction

The somatic cell count (SCC) is widely used for determining subclinical mastitis and evaluate udder health in dairy animals (Berthelot et al., 2006; Dürr et al., 2008). A high SCC is also linked to a loss in milk production and deterioration of milk quality (Gonzalo et al., 1994, 2002; Nudda et al., 2003; Ying et al., 2002, 2004; Leitner et al., 2004a, 2004b; Dürr et al., 2008; Hagnestam-Nielsen et al., 2009; Hand et al., 2012). The electrical conductivity (EC) is one of the indirect systems for determining the quantity of somatic cells in milk (Peris et al., 1991; Barth et al., 2008; Tangorra et al., 2010; Romero et al., 2012). This parameter is widely used for cattle, where the probes for measuring it are often integrated into the milking machine and the EC is continually monitored during milking (Maatje et al., 1992; Zecconi et al., 2004; Norberg, 2005). There are also various portable devices for cattle that by measure of the EC of the milk provide an indication of the SCC (Ferrero et al., 2002). In small ruminants the SCC is usually measured in the bulk tank milk, by laboratory analysis which usually use Fossomatic SCC method. At present there is a portable device for somatic cell counts in ovine milk (DeLaval cell counter, DCC). Swift intervention in sub-clinical and early clinical mastitis before clinical signs appear, and early treatment, has obvious benefits in terms of the yield and quality of the milk and the health of the animals (Milner et al., 1997).

The aim of this study was to design and create a portable device for measuring conductivity specific for Sarda sheep milk. This would be able to monitor the SC level by the EC of the milk of each individual sheep. The receiver operating characteristics (ROC) method was used to identify the EC threshold value that would yield the optimal mix of false positive and false negatives and to evaluate the diagnostic effectiveness of discriminating potentially infected udders in Sarda dairy sheep. The relationships between the EC and the SCC and between the EC and various constituents of the milk were also studied.

2 Materials and methods

2.2.1 Ewe milk characteristics

In the first phase of the study information was obtained on the composition of the milk from Sarda breed sheep. This was before the design and creation of the prototype. A total of 540 samples of half udder milk of 300 ewes were collected before milking and after discarding the first streams of milk (in sterile containers) for analysis from twelve different flocks in the north of Sardinia from February to June 2013. The samples were used to determine the composition of the milk from each individual animal during morning milking. The EC (LF 92, WTW GmbH, Weilheim, Germany), freezing point, chlorides, pH, fat, lactose, protein, (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark), SCC (Fossomatic 5000, Foss Electric, Hillerød, Denmark) of the milk were analysed at the ARA certified laboratory (Associazione Regionale Allevatori) in Oristano (Sardinia, Italy).

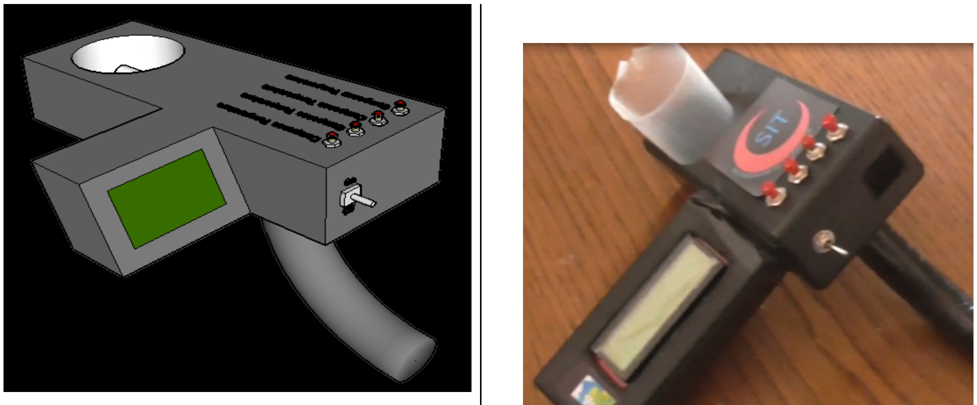
There are marked differences among dairy ruminants with respect to SCC in milk (Souza et al., 2012). The average of SCC threshold values for discriminating between healthy and infected halves differ among species and breeds (Pengov, 2001; Berthelot et al., 2006; Lafi, 2006; Ruegg et al., 2011). Therefore, a threshold of 700,000 cells/ml was set for this study, based on our experience and knowledge in Sarda breed.

The results of the analysis allowed us to identify the EC cutoff value, based on whether the number of cells was greater (or equal too) or less than 700,000 cells/ml.

2.2.2 Prototype design

In this stage a portable prototype was designed and created to determine the SCC from the EC, with a threshold value of 700,000 cells/ml.

The instrument was designed by arriving at a compromise between the differing demands of functionality, precision, speed in taking the samples and cost. Before construction the instrument was modelled in 3D using SketchUp software (version 14.0.4900, 2014) (Figure 8).



a) b)

Figure 8. 3D Model of the portable device for measuring conductivity in Sarda sheep milk (a); the final prototype (b).

The block diagram of the device is shown in Figure 9. The EC probe (k 1.0, Atlas Scientific, NY, United States) was connected to the EC integrated circuit (v 3.0, Atlas Scientific, NY, United States) by a BNC connector. The k 1.0 probe can measure EC in a range from 1.3 mS/cm to 40 mS/cm with a precision of +/-5 µS/cm. The micro-controller used was an ATmega32U4 (Arduino Pro Micro – 5V/16MHz), characterized by: low power consumption, a high performance 8 bit CMOS and low cost. The conductivity readings were compensated for temperature using the DS18B20 (Dallas Semiconductor, Dallas, Texas, United States) digital sensor. This can measure temperatures in a range from -55°C to 125°C with a precision of +/- 0.5°C with a resolution of between 9 and 12 bits, which corresponds to a temperature resolution of 0.5°C, 0.25°C, 0.125°C or 0.0625°C respectively. The temperature of the milk is a critical variable when measuring EC, as an increase in temperature results in greater ionic movement and thus influences the measurement of the EC (Ferrero et al., 2014). Erroneous temperature compensations can result in errors in EC measurements and invalidate the results for the predictive diagnosis of mastitis (Romero et al., 2012 b). The three point calibration of the instrument was carried out following the instructions provided by Atlas Scientific, using two standard buffers (Standardized against NIST-certified references) of 10500 µS and 40000 µS. The accuracy of the calibration of the instrument was tested by comparing the results with those of a commercial EC measuring device (LF 92,WTW GmbH, Weilheim, Germany). The results were more than satisfactory (R^2=0.987).

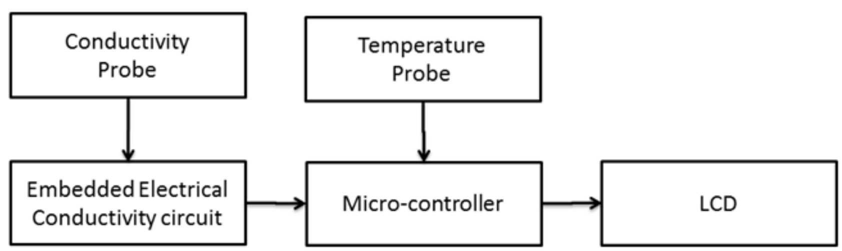


Figure 9. diagram of the device for estimating the SCC in sheep milk

Figure 10 shows the electronic circuit of the instrument. The Rx (receiving) and Tx (transmitting) channels of the EC integrated circuit (v 3.0, Atlas Scientific, NY, United States) are connected, respectively, to pins two and three of the micro-controller. The LCD 16X2 (Sparkfun Electronics, Boulder, Colorado, United States) screen includes an integrated micro-circuit based on PIC 16F88, which allows a serial connection to be made with the micro- controller. The resistance (indicated by R_Onewire) of 4.7 kΩ between the positive pin and signal pin ensures that the DS18B20 temperature probe functions correctly. There are four switches which are normally open (NO): the “Tc” switch for compensating for milk temperature, the “Data Acquisition” switch for reading the EC, the "Calibration" switch for calibrating the device and the "SCC" switch for estimating SCC. The instrument includes a container with a clearly visible stud, which has to be filled with 50 ml of milk so that a correct EC reading is obtained. The firmware loaded in the micro-controller is written in C, the size of the binary file of the sketch is 14.034 kbytes. The device is powered by a 9V battery.

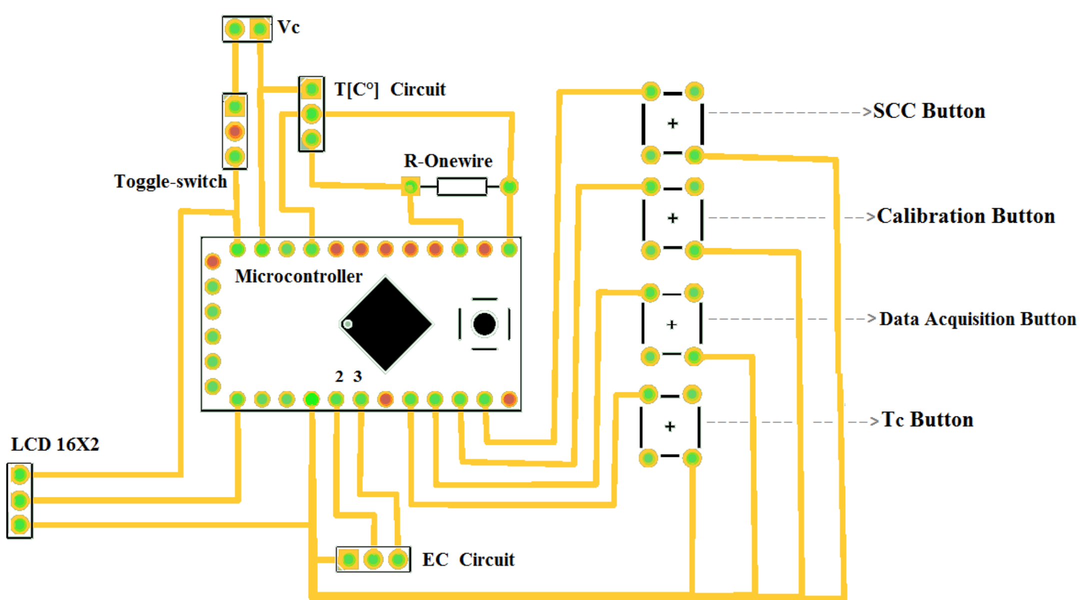


Figure 10. The Printed Circuit Board Layout.

2.2.3 Evaluation of the prototype

In the third phase the prototype was evaluated in the field. A total of 68 half udder milk samples taken at two farms between May and June 2014 were analysed. The prototype was used to measure the EC of the samples directly in the field, and further measurements were then taken in the laboratory, the EC being measured with the LF 92 and the SCC with the Fossomatic 5000.

2.2.4 Statistical analysis

Statistical analyses were carried out using RStudio (version: 0.98.50), and in particular the ROCR (Sing et al., 2005) and pROC libraries (Robin et al., 2011). The values of the different traits measured in the milk are presented as arithmetic mean values and standard deviation. In addition, the Spearman rank correlations between the parameters were calculated.

A non-parametric approach was used to fit the ROC curve to the continuously distributed EC. ROC analysis was used to determine the optimal EC cut-off point for distinguishing between positive (milk with SC ≥700,000 cells/ml) and negative (milk with SC <700,000 cells/ml) results. The point on the ROC curve closest to the top left hand corner was used as the cut-off value. This yields the maximum sensitivity and specificity values and optimizes the rate of true positives whilst minimizing the rate of false positives (Dastjerdi et al., 2013). The sensitivity/specificity pair nearest to the top left hand corner give the most accurate threshold values (Sasse, 2002). The area under the ROC curve (AUC), which can be used to measure the accuracy of the test, was also calculated. A value of 1.0 for the area under the ROC curve indicates that there is a cut-off point for the variable at which there is perfect discrimination between cases and non-cases. A value of 0.5 would be obtained, if discrimination at every cut-off point occurred purely by chance. For an imperfect, but better than casual discriminator, the area under the ROC curve would be in the range 0.5 to 1.0.

2.3 Results and discussion

The average and the standard deviations of the milk parameters under investigation are shown in Table 7.

Table 7. Milk composition of Sarda breed sheep examined in the present study (N= 540)

Item	Mean and standard deviation	Minimum	Maximum
SCC (10^3 cells/ml)	1,144±3,675	26	26,317
Chlorides(mg/l)	143.9±66.26	50.6	693.6
Freezing point (°C)	575.9±13.37	479.0	606.0
Fat (%)	6.45±1.25	2.84	12.52
EC (mS/cm)	4.73±0.54	3.40	7.60
Lactose (%)	4.75±0.50	0.94	5.49
pH	6.59±0.14	5.42	6.91
Protein (%)	5.48±0.67	3.68	11.00

The results for fat, protein, lactose, freezing point and the pH content, all fall within the range reported for Sarda sheep (Nudda et al., 2002). The arithmetic mean of the SCC agrees with the national figures (Rosati et al., 2005), while the mean value of EC was higher than results found by Serra et al. (1997), which ranged from 4.21 to 4.51 mS/cm.

Table 8 shows the values of the correlation coefficients for the variables under examination. The number of somatic cells of the entire sample had a significant positive correlation with the chlorides, fats, proteins and the EC, while there was a significant negative correlation with the freezing point and the lactose. Over all possible combinations of parameters, the highest coefficients were found for EC with chlorides (0.893), lactose with chlorides (-0.843), and lactose with EC (-0.592).

Table 8. Spearman correlation coefficients among milk variables in Sarda breed sheep milk (N=540)

Item	SCC	Chlorides	Freezing point	Fat	EC	Lactose	pH	Protein
SCC	1.000							
Chlorides	.407(**)	1.000						
Freezing point	-.171(**)	-.153(**)	1.000					
Fat	.131(**)	.077	.112(**)	1.000				
EC	.306(**)	.893(**)	-.143(**)	-.216(**)	1.000			
Lactose	-.384(**)	-.843(**)	.232(**)	-.430(**)	-.592(**)	1.000		
pH	.015	-.186(**)	.318(**)	-.209(**)	-.050	.435(**)	1.000	
Protein	.249(**)	.066	.161(**)	.451(**)	-.167(**)	-.343(**)	-.132(**)	1.000

** $P < 0.01$

The correlation between SCC and EC (r=0.306) was lower than that found for cattle (r=0.399; Kasikci et al., 2012), goats (r=0.380; Diaz et al., 2011) and sheep (r= 0.455-0.471; Serra et al., 1997). The freezing point and the EC (r=-0.143) had lower values and opposite signs when compared with the results in studies on cattle (0.228; Kasikci et al., 2012). However no significant difference was found in the correlation between SCC and freezing point in cows (Kasikci et al., 2012), while in our study the opposite was true.

The data on EC were elaborated taking into consideration the number of somatic cells, or rather adopting 700,000 cells/ml as the threshold value for discriminating animals with suspected sub-clinical mastitis (Tab. 9). There were far fewer samples (n=104) with cell values of ≥700,000 cells/ml than samples with values < 700,000 cells/ml (n=436), with the medium values of conductivity of 5.20 and 4.63 mS/cm respectively.

The minimum and maximum levels for both groups showed that conductivity varied greatly. This can be explained by the fact that other factors other than mastitis are related to conductibility (individual variation of EC, farm, parity, and stage of lactation) (Baumgartner et al., 1992; Nudda et al., 2002; Díaz et al., 2011).

Table 9. Descriptive statistics of electrical conductivity (mS/cm) calculated for somatic cell counts of less than 700,000 and more than or equal to 700,000 cells/ml.

	$EC_{SCC< 700\cdot 10^3}$	$EC_{SCC\geq 700\cdot 10^3}$
n	436	104
Mean	4.63	5.20
Median	4.60	5.10
Standard Deviation	0.40	0.69
Minimum	3.40	4.00
Maximum	6.10	7.60

The ROC is one sensitive and specific tool for evaluating the adequacy of a diagnostic test. This allows to identify the best cut-off, or, in other words, the test value which minimises the number of false positives and negatives (Figure 11).

The ROC curve was elaborated from the conductivity data which corresponded to the value of the cells, divided up as in Table 9. The cut-off value (closest top-left index) was 4.835 mS/cm, which corresponds to a sensitivity of 73.08% and a specificity of 75.46% (Tab. 10).

Table 10. Sensitivity, specificity and confidence interval (CI) for coordinates of the ROC curve

Cut off (mS/cm)	Sensitivity %	95% CI[a]	Specificity %	95% CI[a]
4.835	73.08	63.5-81.3	75.46	71.1-79.4

The AUC, which measured the diagnostic accuracy of the test, was 0.804 ($P<0.0001$). This indicated that the test was moderately accurate (Swets J.A., 1988; Greiner et al., 2000) (Tab. 11), or rather indicated that the EC levels were different in the two groups, and thus discriminated sufficiently well between them. In practice, a diagnostic test with an area under the curve of ≥80% is considered adequate (D'Arrigo et al., 2011).

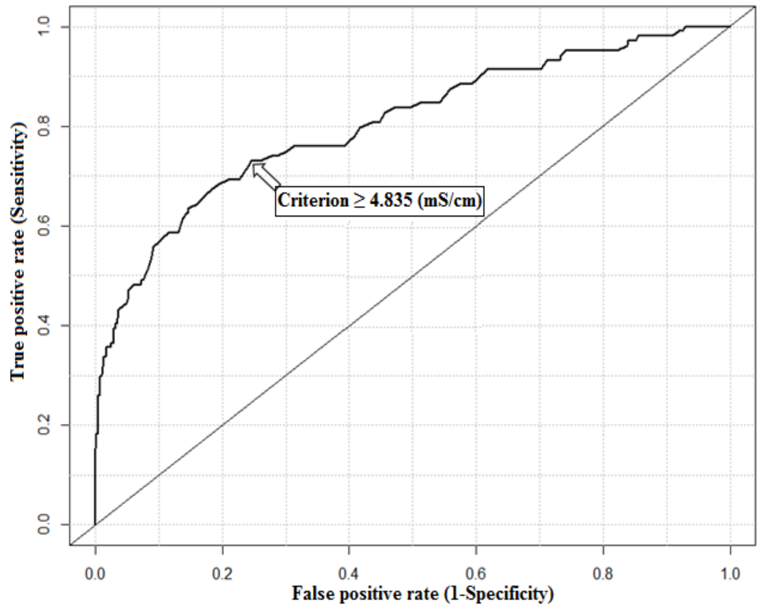


Figure 11. Receiver operating characteristic (ROC) curve between the true positive rate and the false positive rate. The optimal threshold, selected using the closest top-left method, is indicated by the arrow.

The EC was found to be well able to estimate the number of somatic cells, as can be seen by the fact that the confidence interval (CI) of the ROC curve (CI at 95%: 0.768 to 0.837), not included 0.5 (the threshold for diagnostic lack of difference). According to these results, the cut-off value obtained was used in the prototype.

Table 11. Significance level, standard error (SE) and confidence interval (CI) for area under the curve (AUC).

AUC	SE	P	Asymptotic 95% CI	
			Lower bound	Upper bound
0.804	0.0265	<0.0001	0.768	0.837

The flow diagram in Figure 12 shows how the device works. Once the device is switched on, the micro-controller displays a welcoming message on the LCD and then awaits instructions. Once the container integrated in the device is filled with 50 ml of milk, one must press the ‘T_c’ button. This takes the temperature of the milk, which is shown on the LCD screen and is sent to the automatic temperature compensation circuit. The ‘Data Acquisition’ button allows one to measure the EC value of the milk, and this is displayed on the LCD screen and memorised by the device. At this point the ‘SCC’ button, allows the micro-controller to elaborate the EC values, comparing them with the pre-set threshold values (4.835 mS/cm), and showing on the LCD screen whether they are greater than or inferior to 700,000 cells/ml.

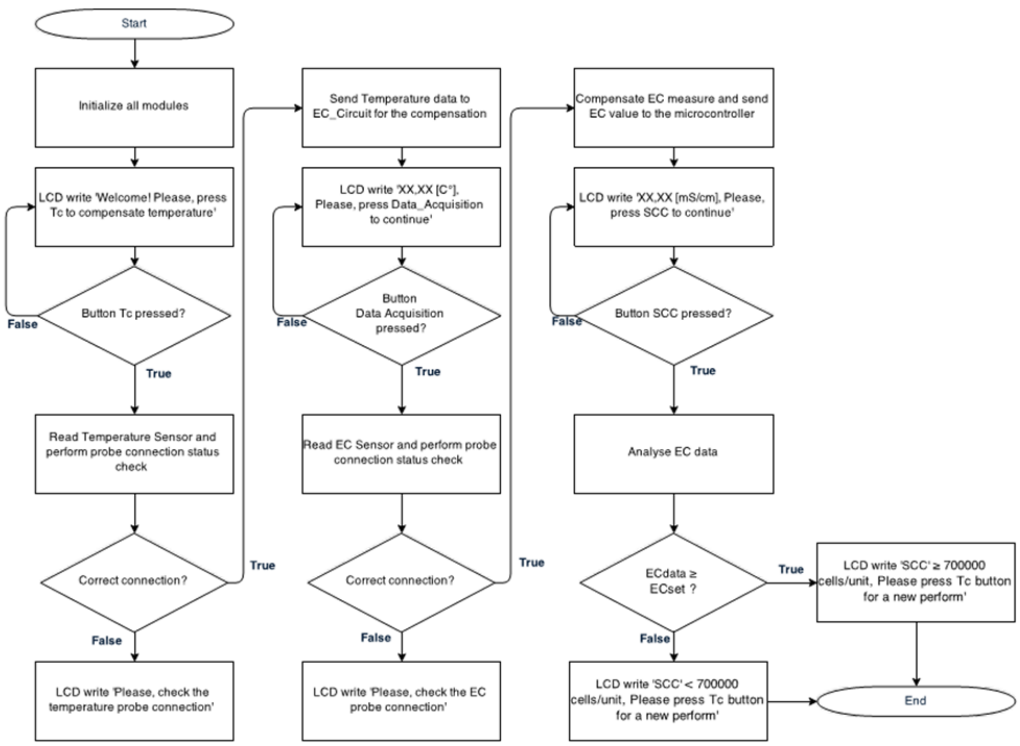


Figure 12. Flowchart of the device.

In the third phase the prototype was tested in the field, and 68 milk samples were analysed (Tab. 12). The device found 11 of the 17 samples which had SCC greater than 700,000 cells/ml (64.7% success rate). When it identified negative samples, its results were confirmed in the laboratory in 39 cases out of 51, with a success rate of 76.5%.

Obviously it is most important to reduce the number of false negatives by as much as possible, as these do not recognise animals which have SCC values greater than the threshold value, and thus the animal itself could suffer because a deeper analysis was not carried out. With our device the number of false negatives was 6 out of 68 samples (8.8%).

Table 12. 2x2 contingency table relating probability of disease status by electrical conductivity (cutoff at EC = 4.835 mS/cm) and disease status predicted from Gold Standard ()

	Expected positive	Expected negative
Positive screening	11 (17)	12
Negative screening	6	39 (51)

2.4 Conclusion

The study showed that measuring the EC is a useful way for identifying sheep with probable sub-clinical mastitis and thus reducing the costs of cyto-bacteriological analyses of the individual milk samples. The portable device described here, specifically designed for sheep milk, gave a good accuracy (73.5%). It allows an initial screening of the SCC to be carried out, based on the threshold value of the EC. Increasing the amount of data available for each animal provides useful information to monitor health status of their udders, and is also helpful when making decisions on the management of the whole flock

3. ANIMAL BODY TEMPERATURE MONITORING: A PRECISION LIVESTOCK FARMING PROTOTYPE APPLICATION

3.1 Introduction

In the last few years the consumer perception and demand are more careful on animal welfare and food quality and safety (Grunert, 2005; Zepeda and Reznickova, 2016; McQuadeet al., 2016). Precision livestock farming (Plf) can provide new opportunities for increasing the efficiency and sustainability of livestock productions and to ensure food safety and animal welfare (Berckmans D., 2014). One of the main aims of the PLF is to provide a support to the farmer in management of the individual animal, using sensors and monitoring techniques that detect the necessary information without subjecting the animals to stress, which could alter the yields and distort the measures (Cangar et al., 2008). In recent years, scientific research has implemented various kind of sensors and data transmission technologies that enable farmers to monitor in real-time various parameters, in order to increase the efficiency of the agro-zootechnical sector. Nowadays the miniaturization of sensors and their low cost can permit to measure in livestock farms both environmental (temperature, air humidity, air velocity, dust concentration, CO_2, CH_4, NH_3) and biological parameters (body temperature, feeding behavior, heart and breathing rate, motor activity, estrous activity, animal's position) (Sevi et al., 2001; Sevi et al., 2012; Berckmans, 2014, Caria et al., 2016). In farms management, for example, is very important the monitoring of animal's body temperature, since its increase is often associated with diseases or productive and physiological disorders: reduction of dry matter intake, reduction in milk production, lower reproductive efficiency (West, 2003). The aim of this study was to provide an easy replicable system able to detect animal's body temperature, using a modular low cost microcontroller, and to send data via Bluetooth to an application loaded into a smart-phone with Android operating system, that allows the visualization and interpretation of measured body temperature.

3.2 Materials and methods

The temperature measuring system was developed trying to contain the costs of materials and at same time identifying the right compromise between materials cost and quality. The four study procedural steps have been (Figure 13): selection and solder of materials, microcontroller programming and Bluetooth module configuretion, Android application (*animal_temp*) programming, testing of the system in laboratory.

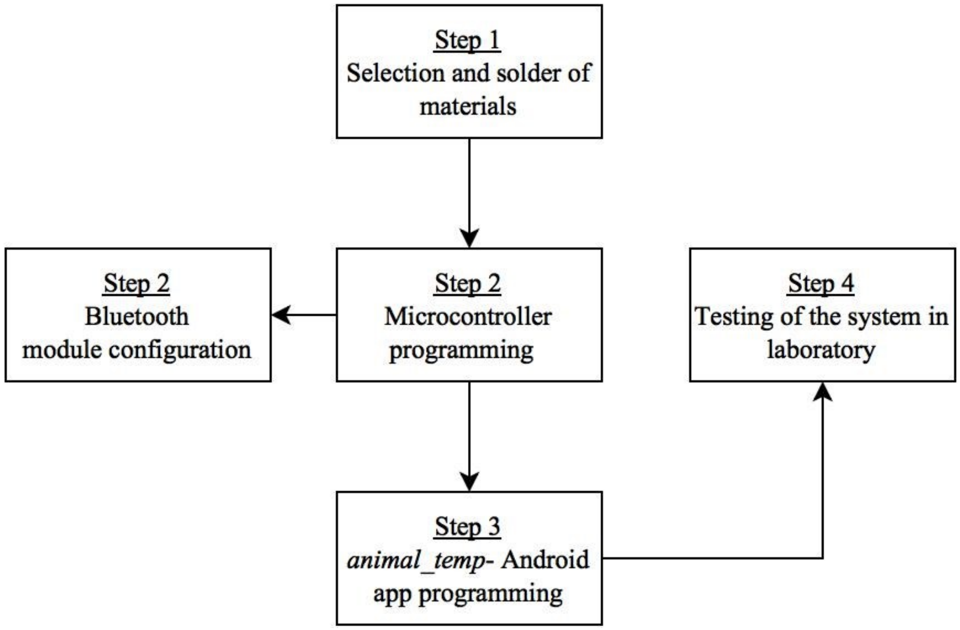


Figure 13. The block diagram of the four procedural steps.

3.2.1 Selection of materials

The microcontroller used was a low cost ATmega32U4 (Arduino Nano V3.0 – 5V/16MHz), with a high performance 8 bit CMOS. The animal body temperature was reading using a LM35D temperature sensor. The LM35D is a precision integrated-circuit temperature sensor with an output voltage linearly which is able to reading temperature from 0°C to 100°C and does not require any external calibration. The sensor was alimentated with single power supplies at 5 V and draws only 60 µA from the supply. The LM35D Transfer Function to convert voltage to temperature is a simple linear equation:

$$V_{out} = \frac{T \cdot V_{supply}}{100}$$

Where V_{out} is the LM35D output voltage, T is the temperature in °C and V_{supply} is the selected supply voltage. The Bluetooth module was a cheapest 2 Class power level and has a build-in 2.4GHz antenna that needn't test antenna. The current in communication is 8 mA and is very small module (27mm×13mm×2mm) with a work temperature range from -25°C to +75°C.The electronic components were soldier in a 2 mm headers breakout board. The electric circuit was designed using anopen source software for Electronic Design Automation, Fritizing Version 0.9.2, developed at the University of Applied Sciences of Potsdam.

3.2.2 Microcontroller programming

The microcontroller hardware has built-in support for serial communication on pins 2 and 3 thanks to a piece of hardware integrated into the chipcalled Universal Asynchronous Receiver-Transmitter (UART). This hardware allows the Atmega chip to receive serial communication from the Bluetooth module even while working on temperature measurement. A library called *SoftwareSerial.h* was upload in the loaded firmware of the microcontroller. The microcontroller is provided of a 10 bit A/D converter, that meaning it has the ability to detect 1,024 (2^{10}) discrete analog levels, that corresponding at 5 v supply a resolution of 4,883 mV, equation 1:

$$V_{pin} = \frac{ADC_{reading} \cdot V_{supply}}{ADC_{resolution}}$$

Where: V_{pin} is the voltage on pin in mV, $ADC_{reading}$ is the A/D converter reading,$ADC_{resolution}$ is 1023bits and $V_{supply}$is equal to 5 V. The temperature sensor lm35d provides an output of 10mV/°C so the equation 2 for extract the temperature from the voltage is:

$$T\ [°C] = \frac{V_{pin}[mV]}{LM35D_{resolution}\left[\frac{mV}{°C}\right]}$$

3.2.3 Android Application animal_temp

The application for smartphone with an Android operating system was write using App Inventor a power software for programming application for Android developed by Google and maintenance by Massachusetts Institute of Technology (MIT). It allows to create software applications for the Android operating system (OS). Nowadays Smartphones have many integrated sensors such as: accelerometer, barcode scanner, clock, gyroscope, GPS, near field, orientation.

3.3 Results and discussion

3.3.1 Microcontroller Firmware

The firmware loaded in the microcontroller is written in C/C ++, the size of the sketch is 6.074 byte.

Global variabels use 339 byte (16%) of dynamic memory, freeing 1.709 byte for local variable. Below the code statements:

```
Import <SoftwareSerial.h>
def variable:
    float tempC
      float averageT
      int tempPin = 0
            for i in range  i < 10
            averageT += analogRead(tempPin)
       delay(20)
      tempC = (5 * averageT * 10) / 1023;
delay(150)
```

```
return tempC
```

3.3.2 Testing of the system in laboratory

Figure 14 shows the electronic circuit of the instrument and the actual prototype. The LM35D sensor was connected on an analogic pin of the microcontroller while the Bluetooth module was connected on pin 2 and 3,which allow the UART communication. The microcontroller was able to provide 5 V/pin but the Rx Bluetooth module operate on a voltage range from 3.1V to 4.2V, so it was necessary to implemented a voltage divider. The voltage divider allows a 3,3 V on the Rx Bluetooth canal, it was composed by using a one resistance of 1 kΩ and one resistance of 2 kΩ.

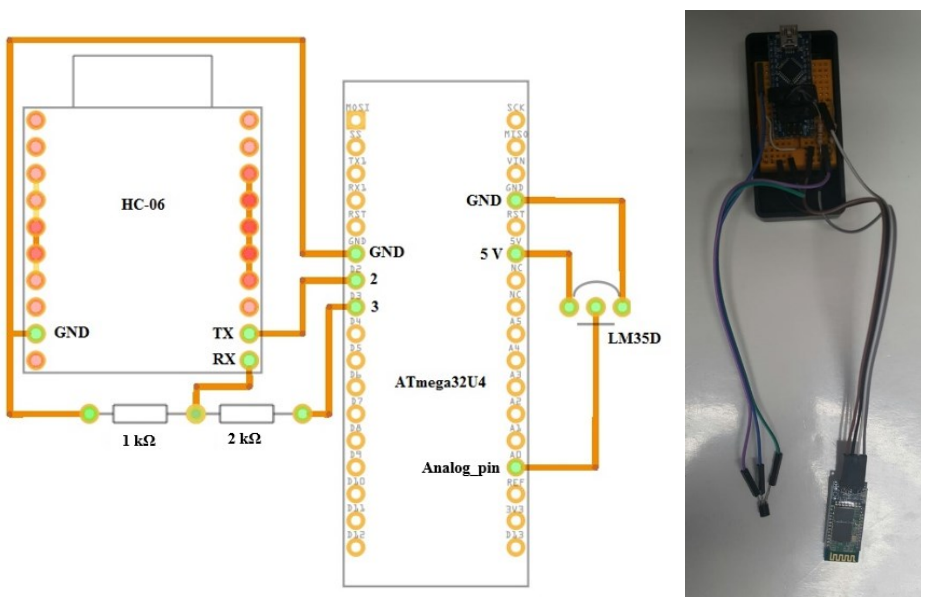


Figure 14. The printed circuit board layout and the actual prototype.

The system was tested in laboratory in a range temperature from 18.8°C to 45.6°C. The system reading temperatures were compared with commercial digital infrared thermometer (KTD-810). The correlation between the reading temperatures was R^2=0.99, in figure 15 the relationship between them.

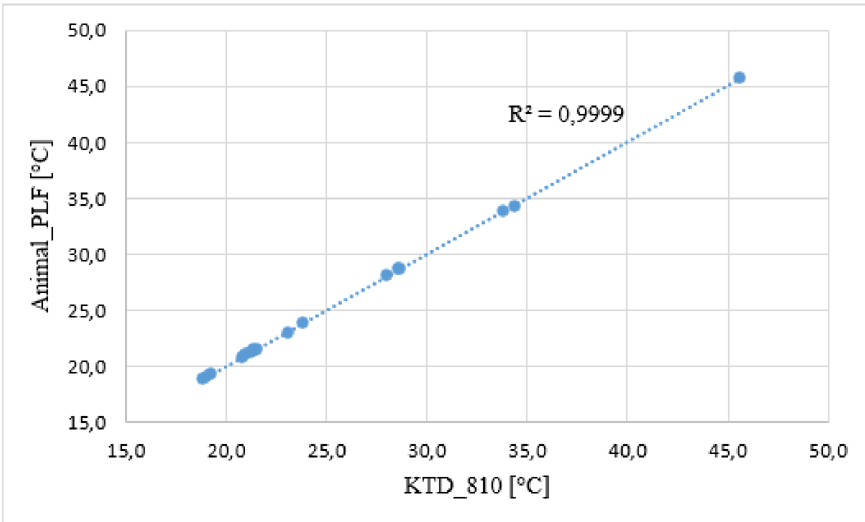


Figure 15. Linear relationship between the temperature reading by the system and the infrared thermometer.

The flow chart in Figure 16 shows how the system works. Once the user opens the app, have to press the button *Bt_connection*, which consent the connexion with the Bluetooth module the value read from the microcontroller was displayed in the display of the smartphone.

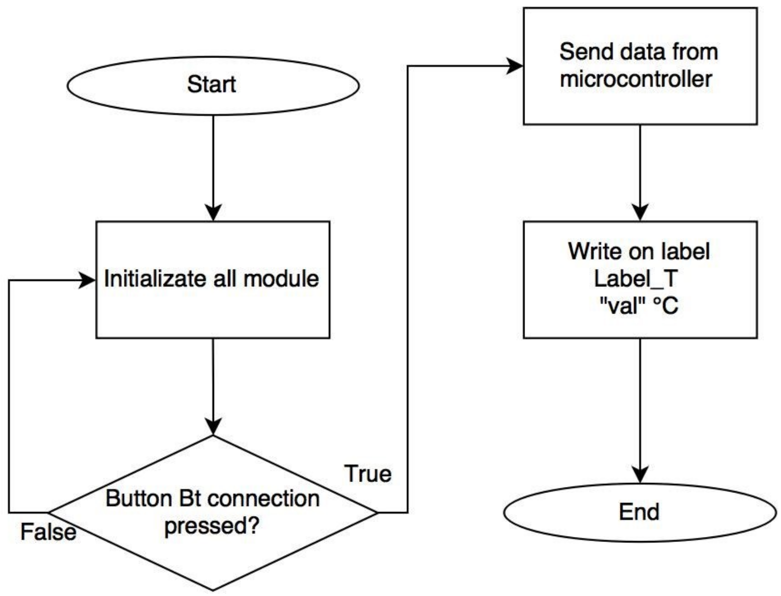


Figure 16. The flowchart of the actual system.

3.4 Conclusions

The study showed a modular and open platform for measuring temperature animal, the many analogic and digital pins present on the microcontroller allow to monitoring many sensor in the same platform with a minimum effort. The recent miniaturization and reduction of production costs of new technologies can provide researchers with innovative tools to overcome the main constraints of the commercial systems provided for the livestock sector, which are the hardware modularity and its closed source software (commercial software or licensed). Many of current's systems present on the market, in fact, allow to add additional sensors and actuators to the system only if they belong to the same trademark, and also the manufacturers often do not offer a complete assortment of sensors for all the measurable parameters in animal farms. Maybe the implemented system can be improved using a 1 class Bluetooth module that allow a data transmission upon to 100~1000 m or different transmission module like Xbee o Wifi. It allows an initial screening of the animal welfare to be carried out, based on the threshold value of the temperature. Increasing the amount of data available for each animal provides useful information to monitor health status of their udders, and is also helpful when making decisions on the management of the whole flock.

4. DEVELOPMENT OF A SOFTWARE PLATFORM, DESKTOP APPLICATION AND ANDROID APP FOR THE OPTIMIZATION OF COLLECTION ROUTES OF GOAT'S MILK

4.1 Introduction

In the recent decades the dairy sector, taking advantage of the intense technological development, has greatly improved its production in terms of quality by increasing awareness on proper sanitary management practices (Bencini et al., 1997); quantity by expanding its technological expertise in cheese production (Smit, 2003); environmental impact by reducing the waste of the production chain (Bell, 2015). The main factors that marked the evolution of the processing industry have been the concentration of production of the establishments, the automation of production processes, the methods to improve product quality and the laboratory techniques to control it (Nuvoli et al., 2013).

The production of sheep milk in the island stands at 330 million liters/year, with an average of 130 liters/head, putting Sardinia in the first place in the sheep milk production among the Italian regions (Idda et al., 2010). According to a survey conducted by the Agency LAORE Sardinia in 2010, the 86 dairies within the regional territory employ 270 tankers to carry out the collection of milk from 15.000 companies. Most of the processing companies employ on average about ten means, the 8% of them uses a much higher number, with a peak of 45 units. From the analysis of collection routes it is pointed out that certain areas of collection overlap each other, while they refer to very distant dairies.

In this scenario, however, it must be acknowledged that the logistics of collection and transport of milk from the company to the dairy have not been studied yet. The improvement of performance in the context of the dairy industries, in fact, has focused on the aspects mentioned above, omitting the organizational side regarding the transport, which not least affects the costs of production.

In the management of shipments or in the handling of goods, especially in the oil and shipping industry, the mathematical models for the optimization of logistics, developed by the Transportation engineering, play a fundamental role in business planning.

In areas where transport is an integral part of the production process but not the heartbeat of the enterprise, such as in the food industry, transport optimization is not always seen as an important management pawn. There is scant regard for logistics, particularly in areas where the cost of transportation is traditionally considered marginal on the overall cost of the service or product. The continuing economic crisis, increasing global competition and stronger environmental awareness of consumers force all sectors of production to a drastic reduction of production costs, including logistics. The mathematical models used for route optimization and fleet planning are characterized by a number of constraints linked to: the staff (daily work schedule, lunch break, overtime, etc.); the transferor or customer (opening times and closing days, loading/unloading times, etc.); to vehicles (capacity of the means, traveling speed, etc.); environmental parameters (road conditions). So theoretically, each activity is logistical unique because each company has its own characteristics, determined by their differences and the territory where it operates.

About the collection of milk, there are still largely insufficient scientific data and information provided to the dairy operators for efficient transport management, which, due to organizational and technical issues related to them, can affect the whole process of transformation (Pazzona, 1988). The constant increase of the costs incurred by the dairies for milk collection from individual producers makes it essential to streamline the collection and transportation system. Furthermore, for geopolitical issues, the dairies tend to get milk from transferors for which the density of collection (l/km) is extremely low, reaching the limits of economic convenience. And besides, the reduction of collection times is important for reasons related to the intrinsic characteristics of the product conveyed, the milk, in fact, is a commodity easily susceptible to the action of bacteria which may cause substantial effects on dairy products obtained from its processing (Salvadori del Prato, 1998).

In the regional territory, specifically, the milk collection optimization for the sheep and goat sector faces challenges related to the considerable fragmentation of companies often accessible through farm roads that do not allow the use of large-capacity tankers ranging from a minimum of 18 t up to 32 t.

This study has been made to test the MilkTour software in a sample dairy, it has been developed by the Territory Engineering Section of the Department of Agriculture of the University of Sassari, and it allows to manage and optimize the stages of harvesting and transport of sheep milk.

4.1.1 The mathematical models of logistics

The algorithms used in the logistics can be grouped into two broad categories: the Vehicle Routing Problem (VRP), which optimizes the management and transport planning, and the Parallel Processing Scheduling (PPS), which streamlines the management and planning of production phases.

The mathematical models for logistics are characterized by constraints that represent the different companies and make them, therefore, difficult to generalize. A classic example of transport logistics is represented by a number of customers, to whom a product / service has to be delivered or picked up (Figure 17).

Figure 17. The figure shows a typical example of a problem related to transportation logistics with 19 customers to whom a product has to be delivered.

This problem can be solved by using an algorithm of the VRP class that, in the specific case of the 19 clients, provides for two rounds with 10 and 09 transferors each (Figure 18).

Typically, the VRP algorithms used are the managerial ones, that is the ones connected to the optimization of transport in situations where a logistical business plan is already present, in which the sequence has been modified. An example of the application of VRP in the management phase is shown in the figure below, where you can see how the sequence of visits of the transferors has been changed by the transport company compared with the solution previously approached. (Figure 19).

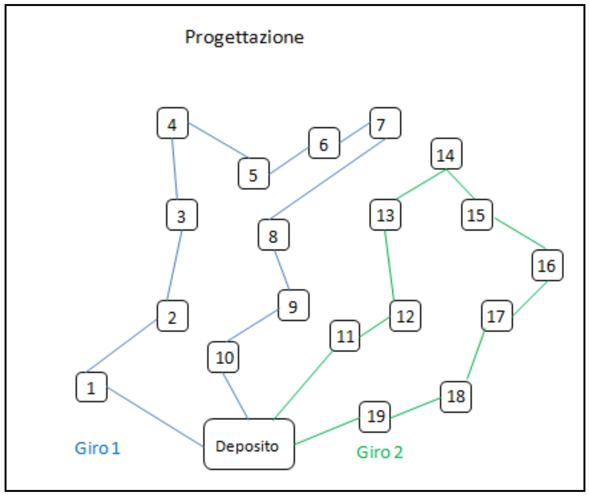


Figure 18. The solution of VRP algorithm in the design phase, where the 19 customers are divided into two groups, visited by two different means or by the same means, but in different periods of time. The first round includes 10 customers as a whole, while in the second they are 9.

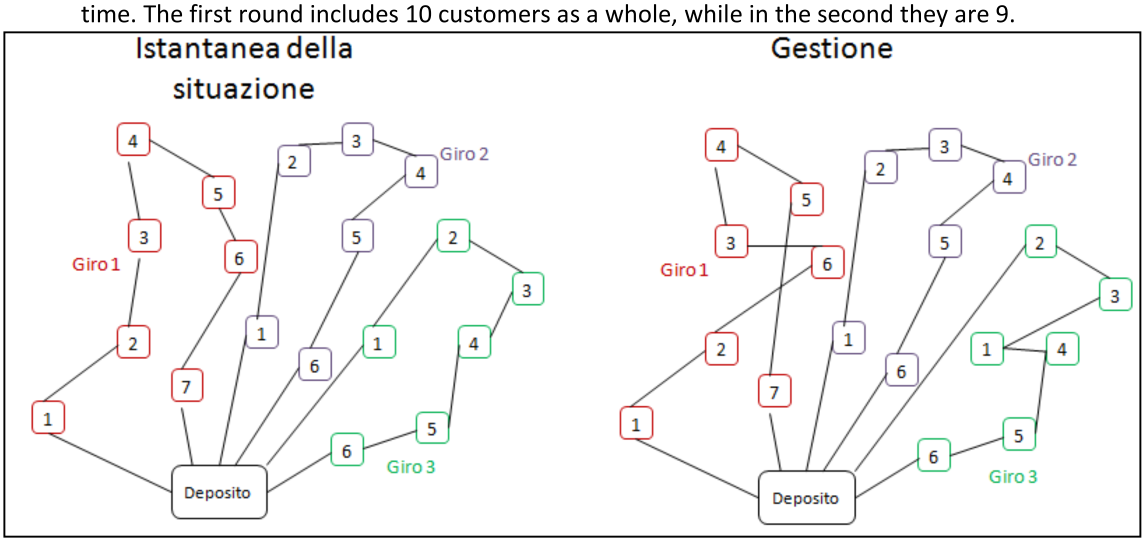


Figure 19. Schematic representation of the transport solution developed by a transport company (on the left). Application of the VRP algorithm with the modified sequence of visits of the transferors (right).

The problems related to logistics can be dealt with "exact" type algorithms or "heuristic" type. The exact algorithms are those that require a high computational calculation guaranteeing the optimal solution. The heuristic algorithms, instead, present a computational order at lower level, that takes much less time to achieve the solutions, but they only guarantee a solution close to the optimal one. The detailed representation of reality within the mathematical models is made possible by the inclusion of constraints that affect the simplicity or complexity of the algorithms.

One of the simplest and most widely used algorithms is definitely the KnapsackProblem, where n data objects, with profit pj and weight wj,, (with j = 1, ... n), and a container (Knapsack) of capacity c, you want to determine the subset of objects j whose total weight does not exceed the capacity of the container c, maximizing the profit. The objective function is the following:

$$x_j = \begin{cases} 1, & with\ j\ selected \\ 0, & otherwise \end{cases}$$

$$\mathrm{Z} = max \sum_{j=1}^{n} p_j \cdot x_j$$

With the contrainst:

$$\sum_{j=1}^{n} w_j \cdot x_j \leq c$$

The simplicity of the algorithm, however, involves a high computational order equal to O(n), making it unusable for a large number of n objects. The Knapsack algorithm can be optimized through the Greedy algorithm, in this case j objects are sorted in descending order from the most affordable to the less convenient according to the relation p_1/w_1 ≥p_(2)/w_2 ≥⋯ ≥ p_n/w_n , thus creating the weights, and packed into the containers up to fill the space. To the complexity of the Knapsack algorithm is added the complexity O(n log n) necessary to sort the weights.

Another model is the Bin PackingProblem(BPP) which takes into account multiple containers (bin) m,each with a capacity c, and the same n objects with weight wj,(j =1…, n). The goal of the BPP is to pack the objects in the fewest possible containers, but in such a way that the sum of the objects assigned to each container does not exceed their capacity.

BPP can be solved in this way:

$$x_j = \begin{cases} 1, & with\ j\ selected \\ 0, & otherwise \end{cases}$$

$$y_i = \begin{cases} 1, & with\ j\ selected \\ 0, & otherwise \end{cases}$$

$$t\ (BPP) = \min \sum_{i=1}^{m} y_i$$

$$\sum_{i=1}^{m} x_{ij} = 1$$

$$\sum_{j=1}^{n} x_{ij} = 1$$

$$\sum_{j=1}^{n} w_j \cdot x_{ij} \leq c y_i$$

The BPP can also be solved with the help of two other algorithms: the NextFitDecreasing and the First FitDecreasing. In both situations, the objects are wrapped one at a time, from the largest to the smallest: in the first algorithm we proceed to package until there is free space, in the second case the object is packaged and placed in the first container having still space available. We can say that in the case of the First FitDecreasing BPP the resource allocation is better than in the first algorithm.

The mathematical models are also applied for the optimization of the production steps, in this context, for example, it may be mentioned the Parallel Processor SchedulingProblem. Imagining of having to program the processing times of some machines placed in series, this algorithm allows to reduce to the maximum the losses in terms of cost, with the goal of having less dead time than the operating time of the machines, so that the production of another unit of product is not allowed during the dead time.

The algorithms previously described are summarized in table 13.

Table 13. The following table summarizes some of the algorithms used for the optimization of

logistics, with the respective formulas and the level of computational complexity..

Algorithm	**Formula**	**Computational complexity**
Knapsack Problem (KP)	$\frac{p_1}{w_1} \geq \frac{p_2}{w_2} \geq \cdots \geq \frac{p_n}{w_n}$ $Z = max \sum_{j=1}^{n} p_j \cdot x_j$ $\sum_{j=1}^{n} w_j \cdot x_j \leq c$	*O(n)*
Algoritmo di Greedy	$\sum_{i=1}^{m} x_{ij} \sum_{j=1}^{n} w_j \cdot x_{ij} \leq c y_i$ $Z = max \sum_{j=1}^{n} p_j \cdot x_j$ $\sum_{j=1} w_j \cdot x_j \leq c$	*O(n)* *O (n log n)*
Bin Packing Problem (BPP)	$t\ (BPP) = \min \sum_{i=1}^{m} y_i$	*O (n log n)*

4.1.2 The mathematical models applied to transportation

The basics of mathematical models applied to transportation must be found in graph theory, born in 1736 with Euler after the solution of the problem of Königsberg bridges. The inhabitants of this town, at the time located in East Prussia, they wondered if it was possible to take a walk along the bridges of the city in order to cross them only once without missing any point. Euler introduced graph theory that proved the negative response to that question.

A graph is a mathematical diagram used to represent the elements of a set and the relationships between them. It consists of a series of nodes or vertices connected to each other with arcs. Mathematically we can describe a graph as an ordered pair T = (V, A) of sets with V vertices set and A arcs set, such that the elements of A are pairs of V elements (figure 20). A graph is defined weighed when it is assigned a numerical value to its arcs.

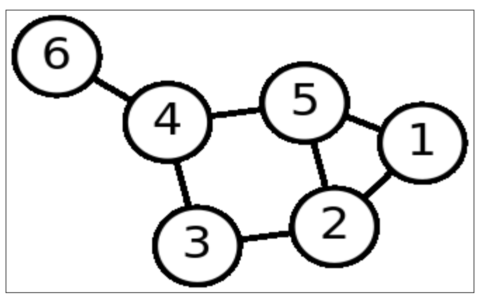


Figure 20. Example of graph with 6 vertices and 7 arcs: the circles represent the vertices, and the numbers inside them the sequence of the visit, the leaders are connected with a line reproducing the arc.

The vertices of the graph represent clients and the arcs the viable ways, which allow us to connect the different clients, so overall the graph can be defined as the mathematical representation of the road network used to carry out the delivery of goods. The graph arcs contain different instructions, which allow to describe in detail the real situation and can be oriented by indicating the direction of travel. It is generally assigned a cost to each arc, the cost typically represents distance and travel time, i.e. the time gap necessary to move along the arc which connects the two consecutive vertices. Each customer is indicated with a vertex and is distinguished for its own characteristics, such as: the geographical coordinates, the quantity of product to give/receive, the time intervals in which he can be served and the time of loading/unloading the goods. The transport of the goods takes place with a fleet of vehicles that can be constituted by means having all the same features, in this case it is referred to as homogeneous fleet, while in the opposite case it is referred to as heterogeneous fleet. All this information allows us to choose what algorithm suits best the case considered. In the case of transport are usually used heuristic algorithms that produce approximately optimal results, since it is tried to find the right compromise between the accuracy of the solution and the time taken to obtain it. By contrast, if there are few knots, or in situations which require a high precision, they are used exact algorithms.

Specifically in the design and/or logistic transport systems management, we talk about a whole class of problems known as the Vehicle Routing Problem (VPR). These are situations in which you need to arrange the routes, vehicles and customers while respecting the constraints imposed by the system. It actually means to reach the goals that can concern other aspects of transport logistics, for example, the minimization of the number of vehicles or CO2 emissions. In the planning and management situation it is commonly necessary to bear in mind the time constraints, better defined as time windows, in which case we talk about Vehicle Routing and SchedulingProblem (VRSP).

The first logistics problem in the transport history is the Travelling Salesman Problem (TSP). Knowing the cities to visit and the distances that exist between them, it is asked to visit them once and only once before returning to the starting city, a typical case of Hamiltonian cycle, along the least-cost route, which can be expressed in terms of mileage or cash. The situation can be represented by a weighted complete graph T = (V, A), whose vertices V = {1,2, ..., n} correspond to all the cities, and arcs A correspond to the roads joining the consecutive vertices, A = {(i, j): i, j∈V}. It is indicated cij with the cost associated to each arc (i, j). In order to be able to analyze the computer with all the vertices we define a binary variable:

$$x_{ij} = \begin{cases} 1 \text{ if the } (i,j) \text{arc belongs to the Hamiltonian circuit} \\ 0 \text{ otherwise} \end{cases}$$ [1]

for *i, j =1 …, n.*

The TSP can be solved by various mathematical models, among others: the two indexes classic formulation, the Miller-Tuckler-Zemlin method, the one-commodity method and the multi-commodity method. Here is the description of the classic two indexes algorithm applied to the TSP resolution.

$$z\ (TSP) = \min \sum_{i=1}^{n} \sum_{j=1}^{n} c_{ij} x_{ij}$$

[1] In a Hamiltonian cycle all the vertices of the graph are touched once and only once.

$\sum_{i=1}^{n} x_{ij} = 1$ con j = 1,…, n

$\sum_{i=1}^{n} x_{ji} = 1$ con j = 1,…,n

$$\sum_{i \in S} \sum_{j \in S} x_{ij} \leq |S| - 1 \qquad \forall S \subseteq V : |S| \leq |V| - 1$$

$x_{ij} \in \{0, 1\}$ $i, j = 1, \ldots, n$

If we use this algorithm to find the least cost path having 10 customers available, the number of possible solutions correspond to 10!, and 3,628,800 solutions, while in the case where the number of customers to visit increases to 70, the number of possible solutions it is 70!, amounting to 1,198 e100. Having a large number of customers, it is clear that the computational effort is definitely very high and therefore it takes time, work and sufficiently powerful machines to calculate and analyze all possible solutions.

The TSP can also be resolved, by reducing the computational load, using a heuristic algorithm, the AntColonyOptimization (ACO). This algorithm belongs to a class of mathematical models that are part of the Swarm Intelligence. It is an approach designed from the Nature Engineering, which is widely used in solving real problems and comes from the observation and modeling of natural phenomena. In particular, the ACO, exploiting the natural character of the ants logistics, allows the optimization of fleet planning and management.

The ACO algorithm emulates the natural attitude of the colony of ants, who starting from their nest indicated by the node N, reach the food indicated by the node C along, in terms of distance, the best way (Figure 21). In the first phase the ants tend to travel distances randomly, until they reach the food, then along the backward route they release pheromones, chemicals released by the exocrine glands of some insects to communicate with individuals of the same species, so as to report to the other ants the road to reach the food. The amount of pheromones released, then the signal strength, is bigger in shorter routes, this allows the colony to choose the best path among the possible ones.

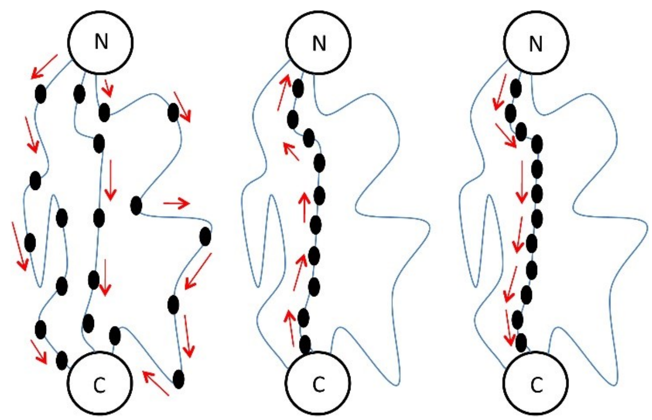


Figure 21. This diagram summarizes the behavior of ants. N indicates the nest, while C with the food that the ants must achieve. For simplicity they are included only 3 routing possibilities. Initially all possible routes are explored, later the ant colony is able to decide, based on the veteran experience, which one is the shortest route.

Below the resolution of the traveling salesman problem with the ACO algorithm:

given a graph G = (V, A) where V is the city ij = (1, ..., n) and A the arcs that connect the city ij, we denote by k the ants who explore the various possible solutions, by visiting in sequence all the cities one and only one time. The amount of pheromone deposited on each Aij arc to iteration t is indicated with gij(t), this quantity is inversely proportional to the distance of the route, it means that in the shortest paths the concentration of pheromone will be bigger. The pheromone values of each arc are updated at each step in order to solve the evaporation phenomenon. After the path Tk(t) the k-th ant releases a quantity of pheromone equal to Dgij(t) of pheromones on each arc (i, j) belonging to the route Tk (t). The probability pkij(t) for an ant k being in the city i to choose to reach the city j depends on 3 factors:

- the fact that the city j has been previously visited,
- the intensity of the measure of desirability to reach the city j that appears to be the inverse of the distance between the city i and j,
- the amount of pheromone released on ij connection.

Taking these factors into consideration, the probability pkij(t) that the k ant chooses the path ij in iteration (t) is equal to:

$$\frac{\left[\gamma_{ij}(t)\right]^{\alpha}[\mu_{il}]^{\beta}}{\sum_{l\in jk(i)}\left[\gamma_{ij}(t)\right]^{\alpha}[\mu_{il}]^{\beta}}$$

if $j \in jk(i)$

equal to 0 if $j \notin jk(i)$

Where a and b are two parameters that can adjust the influence of the pheromones and the coefficient of desirability. In the initial phase it is put a= 0 because all the k ants tend to move in different directions by choosing paths determined by chance, while by setting b = 0 it is described an amplification of the amount of pheromones and in the long period there will be a static situation, where all the ants will walk the same path.

The number of ants used for the resolution of the model is a considerably important parameter: the low number does not allow to obtain the desired results because of the phenomenon of evaporation of the pheromone, but a large number of insects is not very efficient for the system, as it is necessary to analyze a large number of iterations. (Tiboni et al., 2011).

4.2 Materials and Methods

4.2.1 Cooperativa 3A Arborea

The survey was conducted in the dairy of the Pinna brothers (Figure 23), located in Thiesi in the province of Sassari in Meilogu area, in the thirty nuraghi valley.

Figure 23. The Dairy farm of Pinna brothers, located at the entrance of the town of Thiesi (Lat. 40.532165, Long. 8.719546), it covers over 30000 m^2.

At present, every day, 45 refrigerated trucks collect about 260,000 liters of milk produced from 220,000 sheep reared from more than 1,500 companies.

4.2.2 Choice of routes

La In the study they have been analyzed 5 rounds of milk collection from Pinna Brothers dairy, affecting 87 transferors located in the North West of Sardinia, in an area that extends from Planargia to Anglona. The 5 rounds are part of the 45 routes of the Pinna Brothers dairy, geo-referenced by the Section of Land Engineering of the Agriculture Department, University of Sassari. The choice of the sample tours was mainly based on the adjacency and/or overlapping of the areas of collection, so as to be able to better evaluate the functionality of the paths (Figure 24). With a high number of transferors it can be enhanced the potential of MilkTour by modifying the sequence of visits of transferors and identifying alternative routes.

Figure 24. The map extrapolated from MilkTour software includes 87 placeholders, that indicate the same number of transferors of the 5 rounds being investigated.

The milk supplied by the 87 companies is daily collected with the help of five tankers. To the rounds it has been given a unique name for an easy handling of the software: round 1, round 2 round 3 round 4 round 5 (Table 14).

Table 14. Main characteristics of the collection rounds of Pinna Brothers dairy analyzed in this study.

Round name	Transferors (n)	Milk (l)	Route (km)	Collection density (l/km)
Round 1	18	7923	219,59	36,08
Round 2	16	5291	201,92	26,20
Round 3	20	6675	167,29	39,90
Round 4	15	6848	152,22	44,99
Round 5	18	5874	137,78	42,63

Each collection round, 137-219 km long, is composed by 15-20 transferors, while the amount of milk conferred for each round oscillates from 5 to 8 tons approximately. The amount of milk conferred for each round of collection compared to the mileage defines the density of collection. Figures 25-29 show satellite maps produced by the *MilkTour* software.

Figure 25. Satellite map of round 1 of milk collection elaborated by the *MilkTour* software.

Figure 26. Satellite map of round 2 of milk collection elaborated by the MilkTour software.

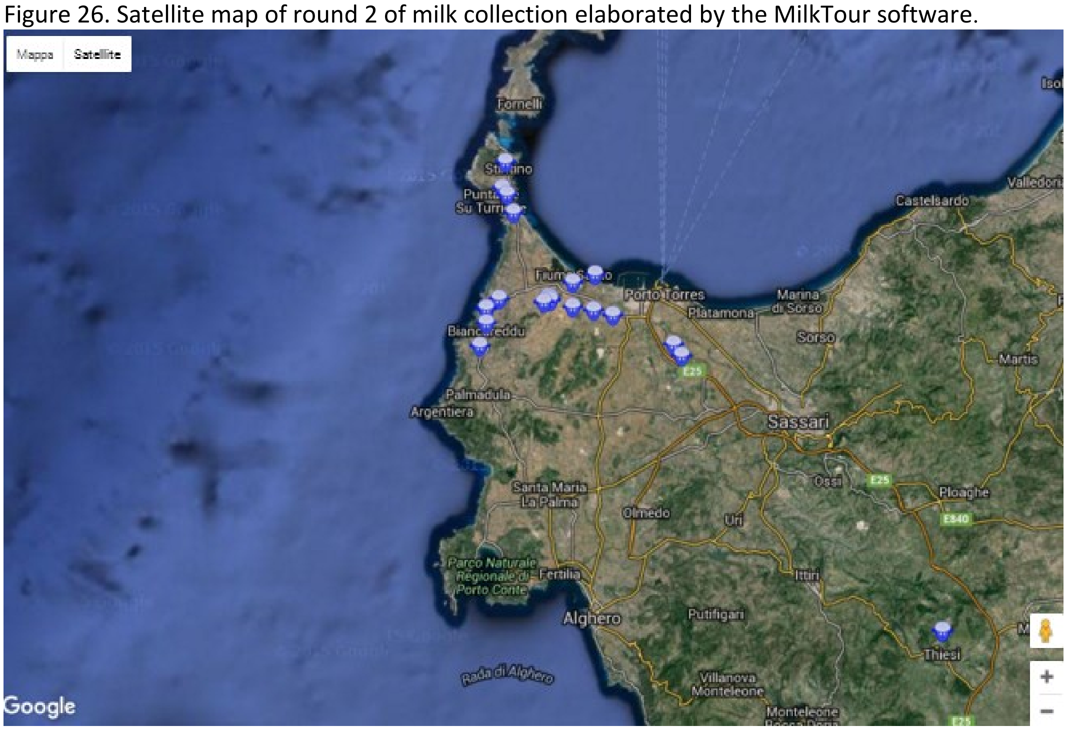


Figure 27. Satellite map of round 3 of milk collection elaborated by the *MilkTour* software.

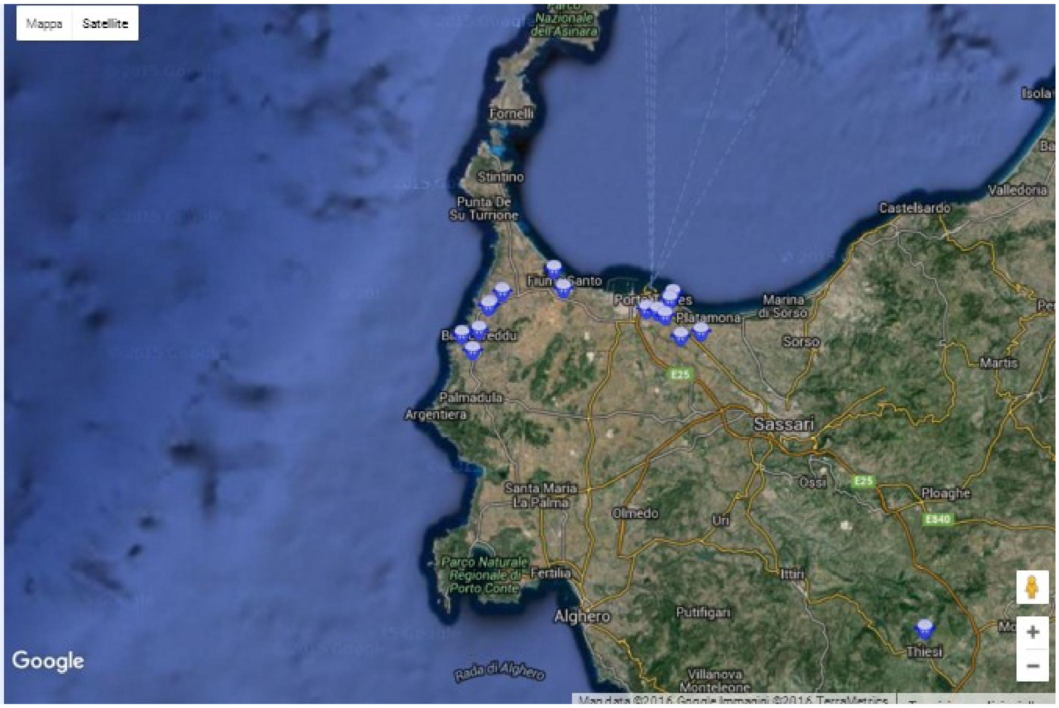


Figure 28.. Satellite map of round 4 of milk collection elaborated by the *MilkTour* software.

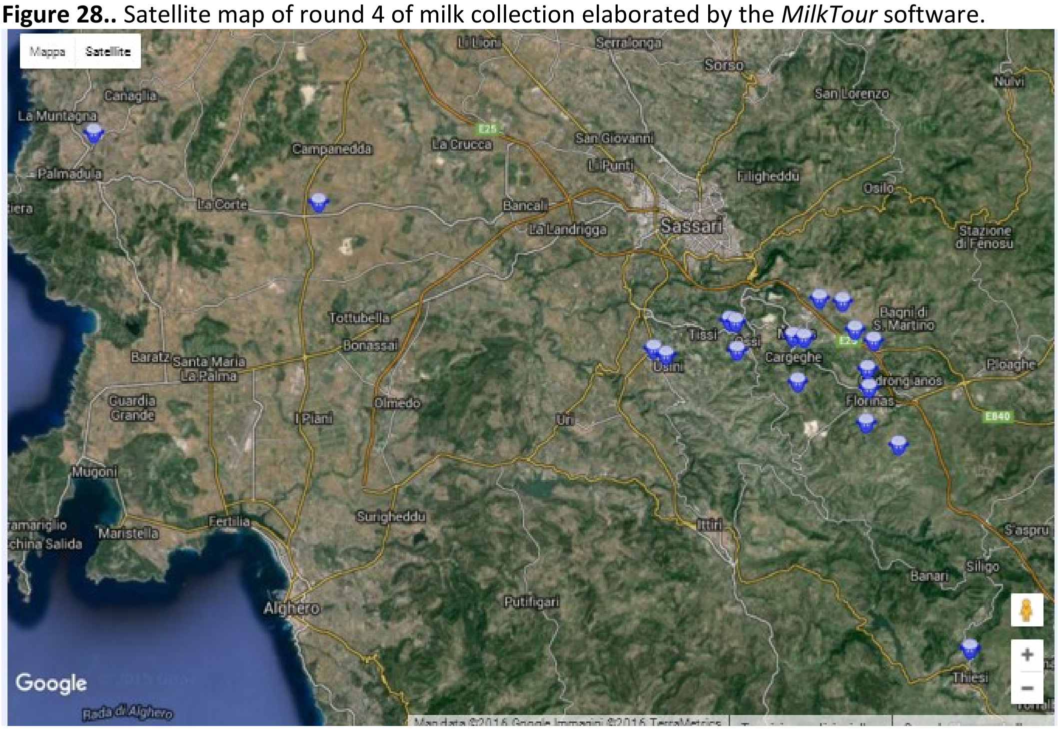


Figure 29.. Satellite map of round 5 of milk collection elaborated by the *MilkTour* software.

4.2.3 GPS Application - Logger for GPS tracks recording

The first step that made it possible to complete the analysis of collection routes was the geo-referencing of the single companies. To detect the unique data of the individual contributor and the route taken by the tanker, the drivers were provided with a smartphone, equipped with GPS antenna, in which it was downloaded the open source application GPS-Logger (Figure 30).

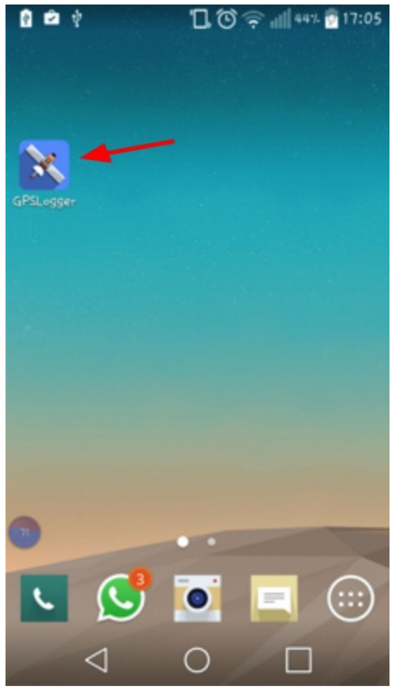


Figure 30. Open-source GPS-Logger application icon, downloaded on the smartphone supplied to the drivers of the tankers. It is a GPS track recorder, simple to use and with low battery consumption, which can be used for the time necessary to complete the route of the milk collection. The application allows to record GPS coordinates, with simple intervals or set by the operator, and then save them in a file on an SD card. With GPS-Logger it is possible to record the time intervals and the distance traveled between a geo-referencing and the successive one.

It is a GPS track recorder, easy and with low battery consumption, which can be used for the time necessary to complete the route of the milk collection. The application allows the recording of GPS coordinates, with simple intervals or set by the operator, and then to save them in a file on an SD card. The application works in background so that the smartphone can be used normally also for other activities. With the use of GPS-Logger it is possible to record the time intervals and the distance traveled between a geo-referencing and the successive one; the data obtained are saved in

GPX and/or KML format and then sent to a predetermined e-mail at time intervals decided at the start, or it is possible to send the data on *DropBox*, *Google Docs* or *OpenStreetMap*.

It is important to remember that the data connection must be present only if it is used the function of auto sending by e-mail or in the case of loading data on *OpenStreetMap* or *DropBox*, in other situations the application is able to store information and send them at a later time.

The drivers of the dairies have been trained on the use of the application in order to obtain standardized geo-referenced data. The application was turned on at the beginning of the round of collection and with the '*Start Logging*' button (Figure 31) each company was geo-referenced. The application provided not only the geographical coordinates, but also the altitude, the date and time of detection.

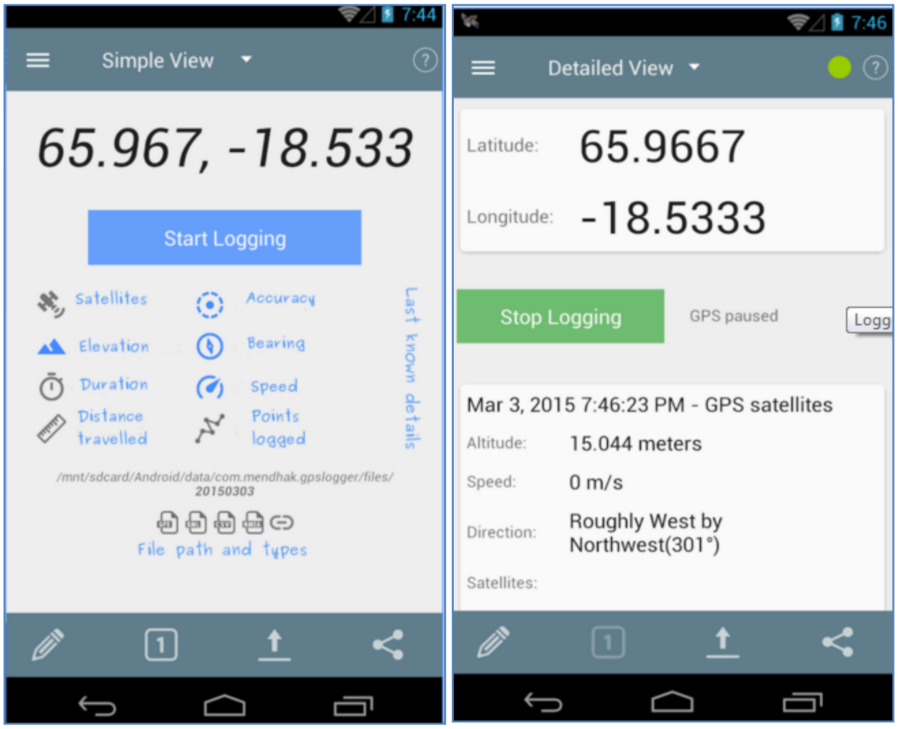


Figure 33. Initial screen of the application '*Open source GPS - Logger*' (on the left) and response screen to georeferencing in which they are shown the geographical coordinates and other information regarding the date, time, and altitude (on the right).

For each round of collection registered, the application returns two files: one with extension .kml (accessible using the free Google Earth) in which it is possible to visually appreciate the distance traveled and the location of the visited transferors marked by the classic Google placeholder (Figure 32), the second contains a .txt file that stores all the information about the round concerned, namely: name and coordinates of the single transferor, start time, mileage, liters of milk collected for each contributor, which in addition to the data extrapolated from information sheets, allowed the creation of a database (.xls) for an easier handling and processing of data collected until now. Since the collection path is subjected to considerable seasonal variability, the data refer to the period corresponding to the peak of lactation.

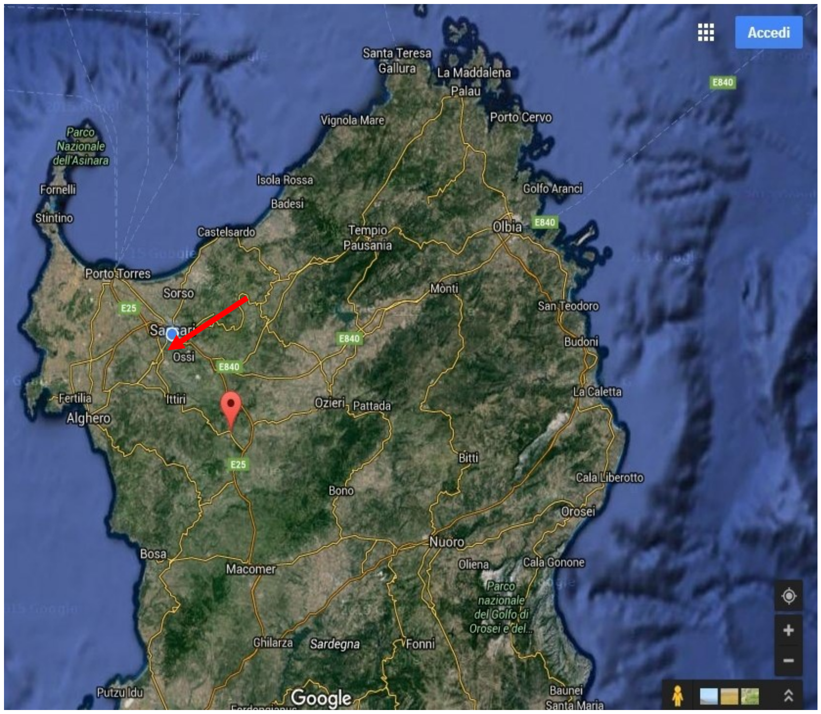


Figure 32. Screenshot of Google Maps with its placeholder. The file is accessible using the free Google Earth.

4.3.4 Software MilkTour for the management of the milk collection

The logistics optimization was realized by using the *MilkTour* software, developed by the Section of Land Engineering of the Department of Agriculture at the University of Sassari, within the project "Analysis of logistics systems and definition of management models for the collection and transport of sheep's milk", financed through the announcement "Promotion of scientific research and technological innovation in Sardinia", regional law 7 August 2007 n. 7. *MilkTour* is a web application developed in PHP language, together with a database for data storing that uses the *Bing Maps* services for both the map view of transferors on the user interface, and for calculating distances in the mathematical model (figure 33).

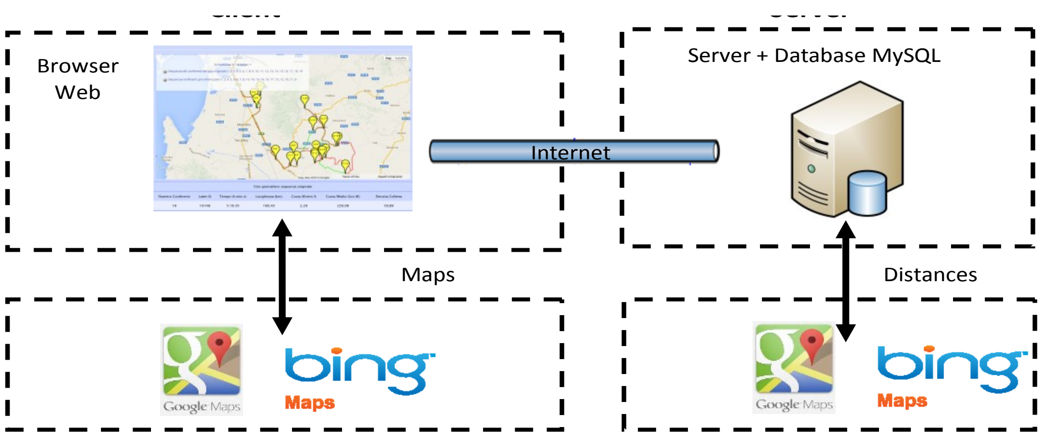


Figure 33. The main interface that uses the free services of Bing Maps for displaying transferors on the map (left). The server that hosts the database, where the geo-referencing of farms are stored, and the mathematical model that uses Bing Maps to calculate distances (right).

From an algorithmic point of view, the issue can be read as a simple traveling salesman problem. The first version of the software tried to solve the problem with the Greedy algorithm, that by analyzing all the possible solutions produces a result quite close to the optimal solution, but after too long time. This mathematical approach was perfect if there were not more than 10 transferors, over this number the result was too far from reality, furthermore it required too much time to get it. While the second, and last, version of the algorithm was based on the ACO algorithm, that allowed to solve the problems that occurred with the previous mathematical model.

The initial screen appears simple and intuitive, it is composed of three elements: a bar, where it is possible to select the collection rounds and costs; a drop-down menu, called "existing collection rounds", through which the rounds present in the database can be viewed, deleted, optimized and dubbed; a third element, "new collection rounds", where it is possible to enter new data previously geo-referenced (Figure 34).

The insertion of coordinates (latitude and longitude) of the new transferors in the software may be done using any spreadsheet, *xls* format, or by downloading the data directly from the *open source GPS-Logger*.

Figure 34. On the top, the bar where it is possible to select two operating windows, one for the collection rounds and one for the costs; on the bottom, the pull-down menu of the collection rounds and on the right the screen where the new data are inserted.

Through the *costs* button on the top of the bar menu, you access the screen to insert the operating costs of the transport vehicle. There are two options:

- Referring to the ministerial costs published by the Ministry of Infrastructure and Transport on a monthly basis. The data in the Annex were elaborated on the basis of the price of diesel detected by the Ministry of Economic Development on a monthly basis, it is a periodic publication of the operating costs of the road haulage on behalf of third parties;

- Taking into account the real costs incurred by the company. In that case you access the screen that allows to enter various items of fixed and variable costs related to travel time of the vehicle in km/year (Figure 35).

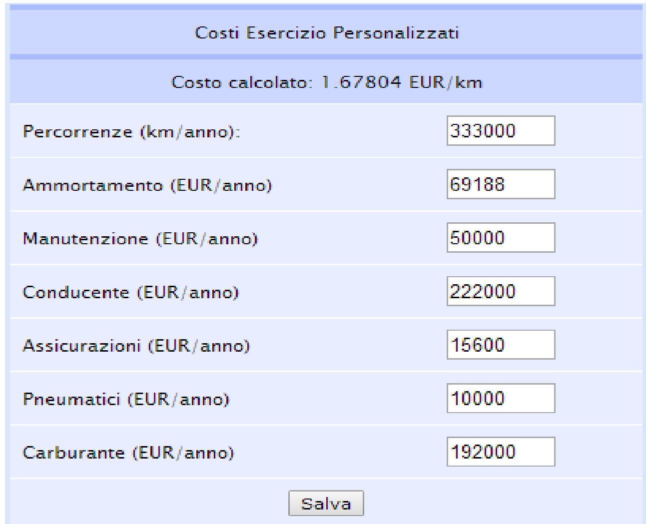


Figure 35. Through the *costs* button on the top of the bar menu, it is accessible the screen that allows to insert the operating costs of the transport vehicle. It is possible to refer to the operating costs of the road haulage on behalf of third parties, published by the Ministry of Infrastructure and Transport on a monthly basis, or take into account the costs actually incurred by the company.

By choosing a collection round, existing in MilkTour database, the user can start analyzing the collection path. The first data that the software provides to the user view are the normal or satellite maps (Figure 36), which provide a clear and immediate idea of the area under study, i.e. the companies visited and the route taken by the tankers to pick up the milk of the transferors.

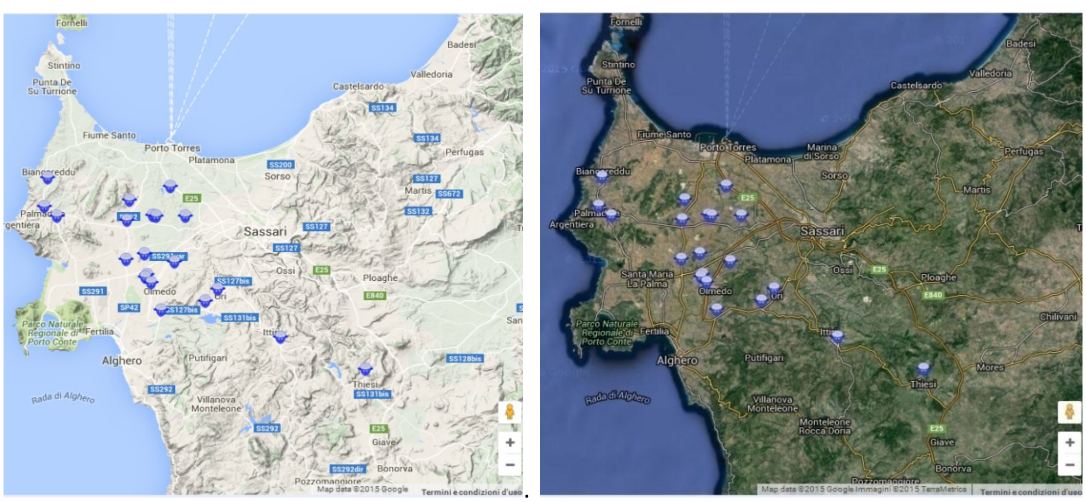


Figure 36. Normal and satellite map of collection rounds, which indicates the enterprises of the transferors and the dairy.

Each farms indicated in the map is shown as a small icon, as well as the dairy, which represents the initial and final stage of the journey. On the bottom, on the same screen, there is a table with the general information of each transferor: the geographical coordinates (latitude and longitude), the liters of milk delivered, the name of the company and location of the same within the collection round.

Furthermore, in the same window of the software, there is the option of searching the minimum cost path assuming to carry out the collection daily, on alternate days or every three days. The user also has the facility to insert constraints such as the maximum dwell time at each company and the start time of the tour. With these data, the program is able to calculate the total travel time which also includes the collection time, i.e. the time required for the operation of decanting and sampling of the milk from the tank to the refrigerant vehicle.

The definition of the minimum-cost path can be achieved through the identification of alternative routes or simply by changing the sequence of visits to transferors. The optimization of collection rounds looks like a typical traveling salesman problem: given a weighted graph, wanting to find the least costly path (total distance or travel time) it is planned to visit all nodes only once and then return to the starting point.

The rationalization of costs should be attributed to the increase of the density of collection derived from the relationship:

$$Density\ of\ collection = \frac{liters\ of\ milk}{km}$$

All these results are shown in the table supplied by MilkTour in the next step after the optimization of routes (Figure 37). The software also provides an immediate indication of the magnitude of savings, resulting from the optimization of the path compared to the original one. A further significant reduction in the cost of collection is always obtained with the collection on alternate days resulting from a doubling of production conferred and, therefore, the density of collection.

Giro Giornaliero Originale							
Giro	Numero Conferenti	Latte (l)	Tempo (H:M:S)	Lunghezza (km)	Costo per Litro (€cent/l)	Costo Medio Giro(€)	Densita Colletta
1	19	7923	4:56:21	147,57	2,59	205,43	53,69

Ottimizzazione limite peso 7923 t												
Dati Generali				Costo Giornaliero				Costo a Giorni Alterni				Risparm
Giro	Conferenti	Tempo (H:M:S)	Lunghezza (km)	Latte (l)	Costo per Litro (€cent/l)	Costo Medio Giro (€)	Densita Colletta (l/km)	Latte (l)	Costo per Litro (€cent/l)	Costo Medio Giro (€)	Densita Colletta (l/km)	%
1	18	4:51:21	147,57	7923	2,59	205,43	53,69	15846	1,45	114,54	107,38	44,24
	18	4:51:21	147,57	7923	-	205,43	-	15846	-	114,54	-	-

Figure 37. Screen of the rounds optimized by MilkTour software: the results are divided between daily and alternate days rounds. The last column analyzes the percentage of savings between the original and the optimized round.

In order to obtain results that are applicable in real life, the software allows you to set some constraints:

• Maximum time available for the round completion, closely related to driver's working time;

• Maximum capacity of the tank.

For all persons engaged in road transport activities, the working hours must provide by law minimum rest periods during the day, amounting to 30 minutes for commitments from 6 to 9 hours. For this reason, the route taken to complete the round should not exceed six hours, even though there are breaks from driving, corresponding to the collection at the suppliers.

Regarding the constraint of the tank capacity, between 8 and 22 tonnes, it is necessary to consider the characteristics of the road system and the space needed for manoeuvre within each company.

4.3 Results and Discussion

Each of the 5 rounds of collection under investigation consists of about 17 transferors and collects on average 6,522 liters of milk per day. To complete a collection round an average of 175 km must be traveled with a density of collection of 37,96 l / km with fluctuations of ± 6,58 l/km (Table 15).

Table 15. Descriptive statistical analysis of the main variables of the 5 rounds of collection investigated.

	Average and standard deviation
Transferors (n/round)	17±1,74
Milk (l/round·day)	6522,20±897,52
Collection route (km)	175,76±30,57
Density of collection (l/km)	37,96±6,58

The time required to complete a round of collection varies from a minimum of 4 to a maximum of 6 hours and the labor productivity is between 0.98 and 1.73 t / h · worker. By adding the distance between the dairy and the first transferor and between the last transferor and the dairy, it has been identified a dead path, by subtracting this result to the total path it was obtained the useful path, i.e. the distance between the first and the last transferor (table 16).

Table 16. Main data related to the 5 rounds currently realized from the dairy.

Round	**Liters**

	(t)	Total time (h)	Labor productivity (t/h·worker)	Route (km)	Density of collection (l/km)
1	7,923	6,04	1,31	219,59	36,08
2	5,291	5,38	0,98	201,92	26,20
3	6,675	4,55	1,47	167,29	39,90
4	6,848	3,95	1,73	152,22	44,99
5	5,874	5,20	1,13	137,78	42,63

The MilkTour software optimized 4 sequences out of 5 by reducing the mileage, this had an effect on the decrease in the cost per liter of milk collected, up to a maximum of 7% to an average saving of € 0.21 (€ cent/l). With the new routes of collection, then, it was realized an annual savings of 3,700 €/round (Table 17). The best results were obtained with the Round 1 in which the route was reduced by 17 km, equivalent to more than 3,600 km in one year, while the largest reduction in the cost per liter of milk supplied was recorded in the Round 2 with a reduction 0.26 € cents/l. The software did not provide any change to the Round 3, probably because it is a path optimized over the years thanks to the experience of the drivers.

Table 17. Comparison between the original rounds of the sample dairy and the rounds optimized by MilkTour software.

Round	Route (km)			Cost (€ cent/l)			Cost (€/round)			CO_2 emitted (equivalent kg)		
	Original	Optimized		Original	Optimized		Original	Optimized		Original	Optimized	
		Round a	*Round b*		*Round a*	*Round b*		*Round a*	*Round b*		*Round a*	*Round b*
1	219,59	182,66	106,25	3,51	3,56	5,61	278,32	205,19	121,19	98,82	82,20	47,81
2	201,92	184,43		4,16	3,90		219,91	206,59		90,86	82,99	
3	167,29	184,31	112,74	2,89	3,73	11,23	192,58	206,50	127,38	75,28	82,94	50,73
4	152,22	143,87	93,66	2,62	2,92	11,30	179,46	171,86	108,78	68,50	64,74	42,15
5	137,78	125,82		2,83	2,63		166,16	154,60		62,00	56,62	

Comparing the reduction in kilometers traveled per round of collection, it is noted how the optimization software affected mainly the dead path, which went from 66% in the original rounds to 47% in the optimized ones (Figure 38).

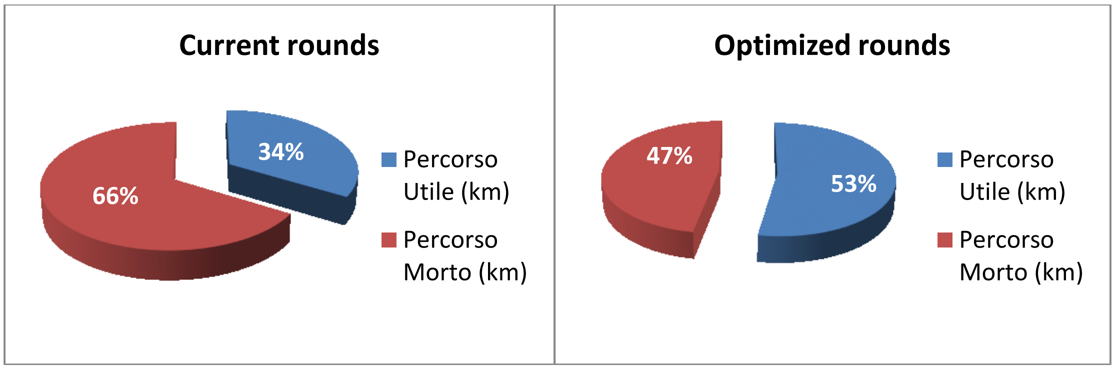


Figure 38. Comparison between the division of the current routes in the dairy and the rounds optimized by MilkTour.

The software has been tested by setting constraints of the tank capacity of 6, 10 and 12 t. In particular, the choice of using a tank of 6 t has a purely demonstrative valence, in fact this type is not present in the vehicle fleet of the studied dairy; in presence of this restriction, MilkTour optimized the various itineraries by dividing them into two rounds, *a* and *b,* where the turns presented a quantity of milk collected higher than six tonnes, in particular in the rounds 1, 3 and 4. The division into two routes determined, obviously, an increase of the costs of collection. In particular, the partition of round 3 extended the path of about 129 km, which means an increase of about 140 €/round, equal to over 29,000 €/year (Table 18). The use of a tank with a lower capacity than the volume of milk that must be collected, determines a considerable additional cost of collecting. Even in terms of CO_2 emissions, the round

3 is the most impactful with 133.67 kg of CO_2 equivalent, in line with the results of kilometers and costs.

Table 18. Optimization of the collection routes with MilkTour, obtained by inserting the limit of tanker capacity of 6 tonnes.

Round	Route (km)			Cost (€/round)			CO_2 emitted (equivalent kg)		
	Original	Optimized		Original	Optimized		Original	Optimized	
		Round a	*Round b*		*Round a*	*Round b*		*Round a*	*Round b*
1	219,59	182,66	106,25	278,32	205,19	121,19	98,82	82,20	47,81
2	201,92	184,43		219,91	206,59		90,86	82,99	
3	167,29	184,31	112,74	192,58	206,50	127,38	75,28	82,94	50,73
4	152,22	143,87	93,66	179,46	171,86	108,78	68,50	64,74	42,15
5	137,78	125,82		166,16	154,60		62,00	56,62	

Applying a limit of 10 tonnes, the software returns an optimization equal to the one without constraints, except the round 5 where there is an increase of about 10 km compared to the optimized round. This increase, less than 10% of the total distance, is widely justified by the fact that the software queries the *Google Maps* server, which is constantly updated about traffic, road closures, etc. The average emission is 98.2 kg of CO_2 equivalent, with a maximum of 114 kg of CO_2 equivalent in round 1 and a minimum of about 78 kg of CO_2 equivalent in round 5 (table 19). It we compare the results of round 3 obtained with the constraint of 6 t and the one with 10 t, we get a reduction of 127 €/round, which translated means more than € 26,500 per year.

Table 19. The data of rounds of collection analyzed with the insertion of the constraints of 6 hours and 10 tonnes.

Round	Route (km)	Cost (€ cent/l)	Cost (€/round)	CO_2 emitted (equivalent kg)
1	200,02	3,28	259,87	114,56
2	184,43	3,90	206,59	105,63
3	185,09	3,10	207,11	106,01

4	151,57	2,61	178,88	86,81
5	136,16	2,80	164,62	77,98

The comparison of the various optimizations with and without constraints is summarized in Figure 39. The tour optimized without constraints is the one that permitted to obtain the lowest cost solution, with € 3.05 cent/l and 197 €/tour.

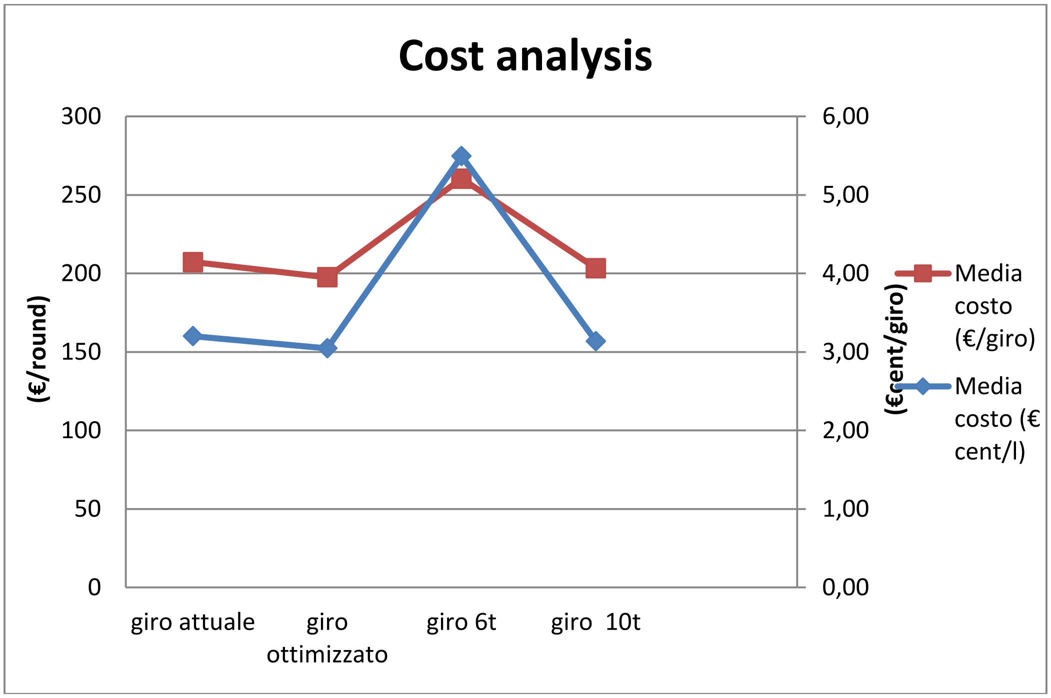


Figure 39. Evolution of the costs per liter of milk collected and per total cost of the tour.

In the last part of the study all the 87 transferors were used to design new rounds of collection. For this purpose, the suppliers were grouped into one group, called by convention single tour, which in total collects 32,771 liters of milk. MilkTour identified a minimum cost path of about 710 km and visits all the companies in a period of more than 23 h (Table 20).

Tabella 20. Characteristics of the tour obtained considering the transferors of the original 5 rounds as members of a single tour.

	single tour

Transferors	87
Milk (l)	32771
Time (h:min:s)	23.23.13
Distance (km)	711,01

This is obviously an unreal and impracticable situation, for this reason the constraints were used to make it feasible. To make the results comparable, the imposed constraints were the same of the previous rounds, i.e. 6 t and 10 t. In the case of 6 t, MilkTour divided the single tour in 6 routes with an average of 14 transferors each. The time taken to complete the rounds of collection varies from 6 h to 6.22 h. The time production varies from 0.70 to 0.90 t/h · worker (Table 21).

Table 21. The description of the 6 rounds identified by MilkTour after insertion of the constraint of tanker capacity of 6 tonnes.

Round N°	Transferors	Milk (t)	Time (h)	Time production (t/h·worker)
1	22	5,525	6,15	0,90
2	17	5,715	6,22	0,92
3	17	5,873	6,00	0,98
4	12	5,922	6,20	0,96
5	7	5,841	6,07	0,96
6	13	3,895	5,53	0,70

The length of the routes varies from 120 to 200 km. The costs per liter of milk collected fluctuate considerably from a minimum of 2.72 € cent/l to a maximum of € 5.78 cents/l, the highest value recorded in round 6 is caused by the value of the density of collection. Also in Table 22, in fact, it is possible to note that the increase of the collection density reduces the cost per liter of milk collected. If they are daily traveled 1030 km with the constraint of 6 t, with the constraint of 10 t they are about 790 km, it means that there is a reduction of 240 km per day which annually correspond to about 50,440 km.

Table 22. The 6 rounds identified by the software after insertion of the constraints 6 t.

Lenght (km)	Cost (€cent/l)	Average cost round (€/round)	Density of collection (l/km)	CO_2 emitted (equivalent kg)
121,22	2,72	150,01	45,58	54,55
177,13	3,51	200,75	32,26	79,71
177,78	3,43	201,75	33,03	80,00
184,27	3,49	206,47	32,14	82,92
163,13	3,24	189,04	35,81	73,41
209,28	5,78	225,23	18,61	94,18

The design from scratch of the single tour, considering a tank with size 10 t, identified the optimal solution in 4 rounds. A larger tank increases the average number of transferors per collection round to about 20. The hourly labor productivity is 0.89 t/h · worker, with a productivity increase of 21% compared to the case of 6 t (table 23).

Table 23. Description of the 4 rounds identified by MilkTour after insertion of the constraint 10 t.

Round N°	Transferors	Milk (t)	Time (h)	Time production (t/h)
1	23	5,945	6,72	0,89
2	18	9,875	6,42	1,54
3	19	9,756	6,28	1,55
4	18	7,195	7,52	0,96

By analyzing the data obtained, there is a reduction of the cost per liter of milk collected, in fact the highest value is € 4.58 cents/l against the € 5.78cents/l obtained in the constraint 6 t, that is a reduction of 1,2 € cents/l. Also in this case the highest cost for liter of milk collected is obtained in the round with the lowest density of collection. Among the 4 rounds identified by the software, we can see that the first

3 have been optimized while the fourth is characterized by high costs, low density of collection and higher CO_2 emissions (Table 24).

Table 24. The 4 rounds identified by the software after insertion of the constraint 10 t.

Lenght (km)	Cost (€cent/l)	Average cost round (€/round)	Density of collection (l/km)	CO_2 emitted (equivalent kg)
152,6	3,02	179,8	38,96	87,40
178,95	2,42	238,94	55,18	102,49
182,15	2,48	242,2	53,56	104,32
278,89	4,58	329,2	25,8	159,73

A further important result is provided by the correlation between the density of collection and the cost per liter of milk collected equal to 0.94. The equation that best describes the relationship between these two variables is the exponential type (Figure 42):

$$y = 7.5884e^{-0.023x}$$

Given this strong correlation between density and cost it is possible to make a first judgment of economic convenience of a round of collection and its respective optimized round by comparing the density of collection.

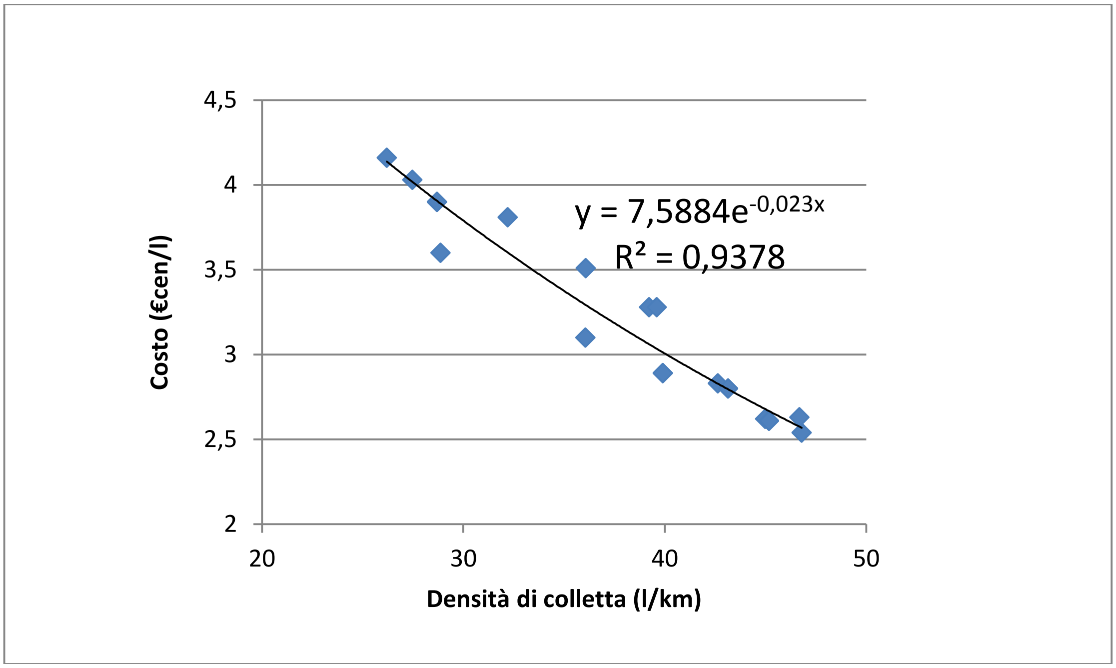


Figure 42. Evolution of the cost of milk collection on the basis of density of collection.

4.4 Conclusions

This study highlighted the importance of optimizing the collection routes as they can significantly affect the final cost of the product incurred by the dairy processing centers.

The rationalization of the collection system of sheep milk in this work represented a first approach to the problem of planning the milk transport. The least cost solution found by the software was obtained by minimizing kilometers of dead path and changing the sequence of visit of transferors. In the 5 rounds analyzed the dead path was reduced on an average of 19% , with a peak in the 5 th round of 77%, equivalent to about 89 km.

The reduced number of kilometers allowed to gain, in terms of € cents / l, an average of 5%, with a peak of around 7% in round 1, a saving amounting to around € 3,800 annual out of 7923 l. Considering the 330 million liters of sheep's milk collected in 650 collection routes, it is assumed a large degree of optimization and profit.

A further important contribution that emerged, is the strong correlation between density of collection and the cost per liter of milk collected (€ cent/l), which detects the cost-effectiveness of a round of collection and its respective optimized round.

5. DESIGN OF A PORTABLE INSTRUMENT FOR THE ESTIMATE OF SOMATIC CELLS BY Na+

5.1 Introduction

A marker that monitors the udder health status in dairy ruminants is represented by the somatic cell count (SCC) (McDermott et al., 1982; Dohoo and Leslie, 1991). In the SCC they are included both the blood-derived cells and epithelial cells, the latter are normally present in the milk and do not represent a pathological aspect (Poutrel, 1981). In a situation of non-specific malaise the immune system calls in the udder, from blood, white blood cells, and the milk registers substantial increase of SCC, for example: infections by microorganisms (Pengov, 2001; Ariznabarreta et al., 2002), shock and/or lesions of the breast tissue (Burriel, 1997), incorrect milking (Pazzona et al., 1993), abrupt nutrition changes (Pulido et al. 2006) and environmental stress (Collier et al., 1982). Intramammary infections (IMI) in dairy sheep represent a risk to the company's profitability both in terms of production (Gonzalo et al., 2002; Leitner, 2008) and health, as the sheep's milk is mainly used for cheese making (Pirisi et al., 2000; Leitner et al., 2003, 2004;-Ljutovac Raynal et al., 2007). The SCC is a significant variable for identifying the IMI, either manifest or not, several studies have deepened, in the sheep sector, a link between the IMI and the SCC indicating considerable variability in defining a possible absolute threshold value to discriminate a healthy animal from an infected one. The bibliographical references (Table 25) indicate wide physiological values, from a few hundred to over one million SCC, although many authors identify this value below 500 x10^{3}cell / ml (Gonzalo et al., 2002; Tolone et al., 2014; Leitner et al., 2004; Boselli 2004; Pengov, 2001; González-RodríGuez et al., 1995; Tolone et al., 2014).

Table 25. Percentage of isolation of the organism responsible of IMI, prevalence of non-infected samples and SCC of various races

Breed	Sample	Log SCC		Isolates %	Prevalence %	Literature
		Uninfected	Infected			
Valle del Belice	14072	5.17	5.41 (5.74)	0.6 (80.51)	55.89	Tolone et al., 2014
Israeli-Assaf Israeli-Awassi	1630	5.00	5.69 (6.11)	26.3 (66.79)	68.00	Leitner et al., 2004
Comisana	1827	5.75	(6.11)	0 (75.23)	87.95	Boselli et al., 2004
Sarda	1827	5.68	(6.12)			Boselli et al., 2004
Sarda	586	5.31	5.94	0 (77.5)	75.8	Pirisi et al., 2000
Assaf	686	5,18	6,15	58(445)	64,87	González-RodríGuez et al., 1995
Castellana	274	5,07	6,1	63,7(113)	-	González-RodríGuez et al., 1995
Churra	9592	4.98	5.97	51,63	75,36	Gonzalo et al., 2002
Domestic highland, East Friesland and Awasi	496	4.95	(6.28)	26.15 (73.85)	73.8	Pengov, 2001
Valle del Belice	14072	5,17	5,74	2(85)	7865	Tolone et al., 2014

() Coagulase-Negative Staphylococci

Several factors influence the variability of SCC in sheep: the breed, the parity degree, the stage of lactation, the heat (Gonzalo et al., 1994, 2002, 2005; Lafi et al., 1998), but for equal breed, that in the analysis of variance carried out by González-Rodriguez et al., 1995, represents the second variable, after the IMI, able to explain the variability of the SCC, the hemi-udder factor together with the residual error, explains in the analysis of variance of the SCC into the milk, carried out by Gonzalo et.al, 1994, the 83.2% (P <0.001). Furthermore, several studies identified among the minor pathogens, in coagulase-negative staphylococci (CNS), the most isolated bacterium during the IMI for different breeds of sheep (Table 25). The CNS are primarily responsible for the not manifest IMI, while other pathogens have lower values in low SCC and in some cases even lower for healthy animals and also with very low isolation percentages (MicrococcusluteusLogSCC infected 4.65 ± 0.29; LogSCC uninfected 4.86 ± 0.52 with a percentage of isolation equal to 0.2%). In any case the SCC is difficult to implement on a large scale due to the laboratory instrumentation costs (McDougall et al., 2001).

An indirect method[2] for estimating the SCC is the measurement of electrical conductivity (EC) for which there are commercially appropriate EC readers both online and portable. Unfortunately, the correlation between EC and SCC, in the sheep sector (R^2 = 0.306; Caria et al., 2016) is lower than in the beef sector (R^2 = 0:39; Lafi et al., 2004). The EC in milk depends on several factors including the ion concentration (Na^+, K^+, Cl^- and Ca^{2+}): in particular during an IMI the content of lactose, main osmotic component, decreases as the synthetic function of the mammary gland decreases, calling by the immune system, due to the rupture of tight

[2]Current and potential 'on-line' assays for the detection of mastitis in milk. The colour, electroconductivity and the SCC sensors are currently used as 'on-line' assays, whereas the sensors for the detection of NAGase, haptoglobin and gases produced by bacteria have yet to be incorporated 'on-line'. These sensors show great potential for the accurate detection of mastitis (Viguier et al., 2009).

junction, the white blood cells from the blood vessels present in the udder, but also sodium ions, chlorine and potassium in order to maintain the osmotic balance (Raynal-Ljutovac et al., 2007). Furthermore, the variation of mineral concentration in milk in a healthy udder plays an important role in the attitude of dairy milk (Pirisi et al., 2000). The study aims to:

- provide a preliminary indication of the physiological values of NA + of the Sarda breed sheep's milk, and highlight the correlation with some of its physical-chemical indices and, in particular with the SCC and the EC;
- design a portable and low cost reader, specific for sheep, able to make the early diagnosis of subclinical mastitis by the measurement of the Na + ions in the milk.

5.2 Materials and methods

The objective of the first phase was collecting information on the composition of the milk of Sarda sheep breed. A total of 2,000 milk samples were taken from hemi-udder in sterile containers for the analyzes (aseptic) in three different farms in the "region" of northern Sardinia. The samples were collected during the morning milking, in the period between March and June 2014. Milks were analyzed for EC (WTW LF 92), freezing point, Chlorides, pH, fat, lactose, protein, (Milkoscan FT 6000, Foss Electric, Hillerod, Denmark), somatic cell count (SCC) (Fossomatic 5000, Foss electric, Hillerød, Denmark) at the certified laboratory ARA (RBA- Regional Breeders Association) of Oristano (Sardinia, Italy). In the second phase of the experiment it was designed a portable prototype for the estimation of somatic cells in sheep milk. The results obtained in the first phase allowed to determine an optimal algorithm based on NA + ions able to make the best discrimination of samples with a high content of SCC.

5.3 Description of the prototype

The Device

The instrument incorporates two containers which receive and analyze milk samples taken from emimammelle simultaneously (Figure 43 & 44). The milk containers have such a width as to allow easy cleaning of the same, to be carried out with running water, between a measurement and the other. Each container has integrated in its interior a pair of sensors that detects the value of Na + of the milk. The containers are marked by a red mark that indicates the minimum volume of the sample that should be taken in order to make a correct reading of Na^+.

Near each container there are placed two colored LEDs, one red and one green, which allow instant viewing of diagnostic prediction of subclinical mastitis, with the lighting of the green LED in case of regular milk and red vice versa in the presence of milk with a high content of somatic cells. The tool also comes with a slot for inserting a memory card (SD), which allows storage of the data, this allows the breeder temporal comparison of measurements on individual garments for emimammella. The device is easy to use since it comes with three buttons that allow you to: display the temperature of the milk, the Na + and the data stored in the SD.

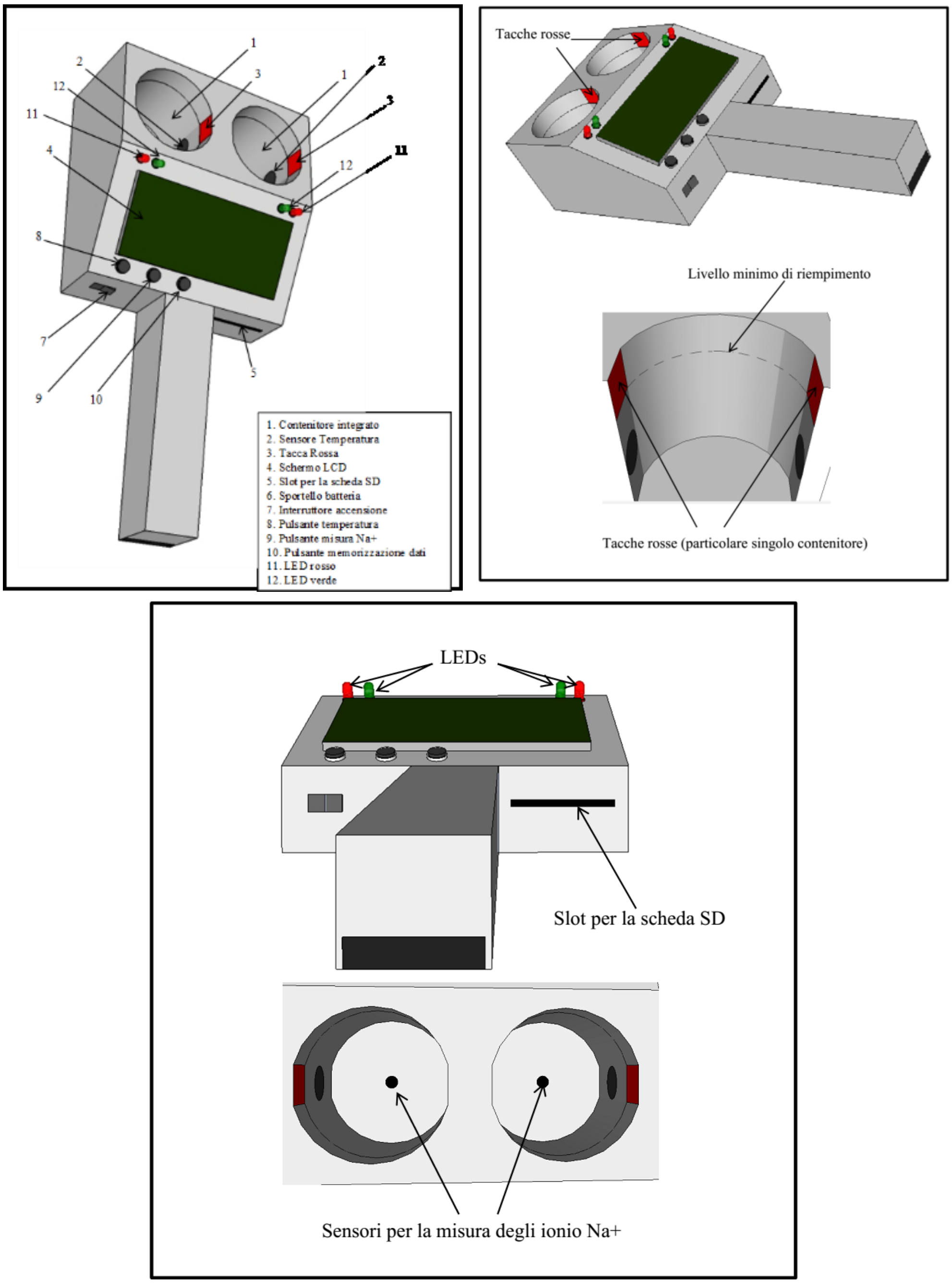


Figure 43 Overall view of the device for predicting the diagnosis of subclinical mastitis in sheep. The pairs of red notches, between their mirror, are located within each container and indicate the minimum quantity of milk to be taken in order to properly measure the Na + ions.

The stored data can be downloaded into a PC or display on the LCD screen, integrated into the device, which allows the display of the value of Na + and the temperature of the milk of both containers.

Milk containers

An important aspect of the device is given by the integration of the double container that allows simultaneous measurement of milk taken by two emimammelle the same animal, thus simplifying the cataloging of data on the SD card. Furthermore, the cylindrical containers are quite large (40 mm) diameter to allow for their easy cleaning between measurements and the other. The height of the containers is equal to 40 mm of which 10 mm of the upper part each marked by two red marks which indicate the minimum limit of filling of the same, equal to 37 ml. This quantity is representative of the entire milk sample and allows the immersion of the Na + sensor placed in the container bottom.

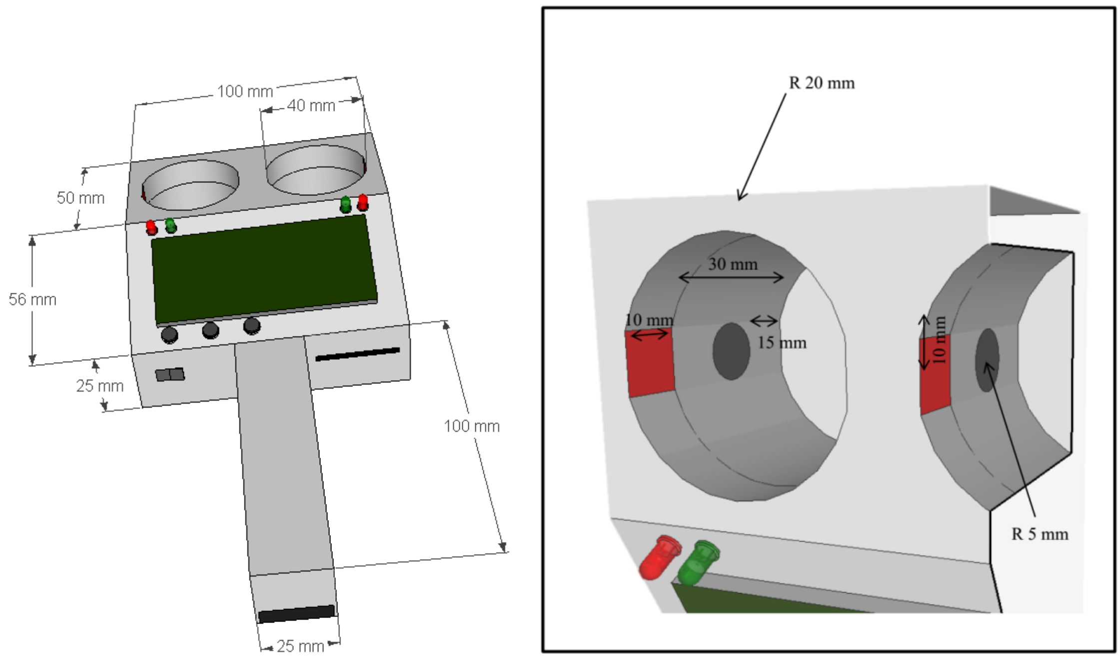


Figure 44 The double-box configuretion accelerate and simplify the process of measuring the Na + ions creating a robust device, sealed and easy to clean.

Operating modes of conduction of the device

The portable device is powered by a 9-volt battery, which is allocated into the end of the handle in a special compartment in order to receive a certain protection from dust and liquids. The device is equipped with an ignition switch, a slot for access to the SD memory card, an LCD screen, four LEDs and three buttons whose function is to: detect the temperature of the milk, measuring the EC and store the size performed. The electronic parts are placed inside the casing of the device, mounted on a plate that includes a printed circuit board and the electronic components necessary for operation.

Laboratory tests have identified the threshold value for predicting infection mastitis diagnosis (unpublished data). The microcontroller turns on the red light or the green depending on whether the Na + of the milk sample is respectively greater or less than the predicted threshold value. The use of the device is as follows: the operator after connecting the 9-volt battery, switch the power switch to viewing the LCD display a welcome message. Before milking, the operator, after positioning the device under the breast, fills the container up to the top of the appropriate red tacchete that delimit the right amount of milk for the correct measurement of the Na +. The operator after pressing the measurement of Na + button, automatically temperature compensated by the microcontroller, immediately detects any presence of infection by the glowing red LED and performs the reading on the LCD screen of the Na + values in mg / ml or ppm. The tool also can monitor the milk temperature for both containers by selecting the appropriate button that is displayed on the LCD display in degrees Celsius (° C). The button for the data storage, finally, allows you to save the data on the SD card. After the measurement, it eliminates the milk present in the containers, which are rinsed with potable water, and go to the next measurement.

5.4 Results and discussion

Diagram of the device

The portable device includes a microcontroller, a shield for the SD card, two red LEDs, two green LEDs, an LCD screen 16x2, a temperature sensor, a 1 ° of the signal amplifying stage (operational amplifier in noninverting configuretion), a 2 ° of the signal amplified offset stage (operational amplifier in differential configuretion) and the sodium ion sensor which is formed by a selective membrane of ions (by dissolving 0.07g of PVC and 0.15 g of tetra-alkylammonium salt salicylate in 2.0 ml of THF). The electrode body must be filled with a solution equal to 0.01M NaCl and 0.10M of sodium salicylate. By applying a positive potential to the silver wire with respect to a platinum wire in a HCl aqueous solution it is possible to measure the sodium ions present in a liquid (figure 45).

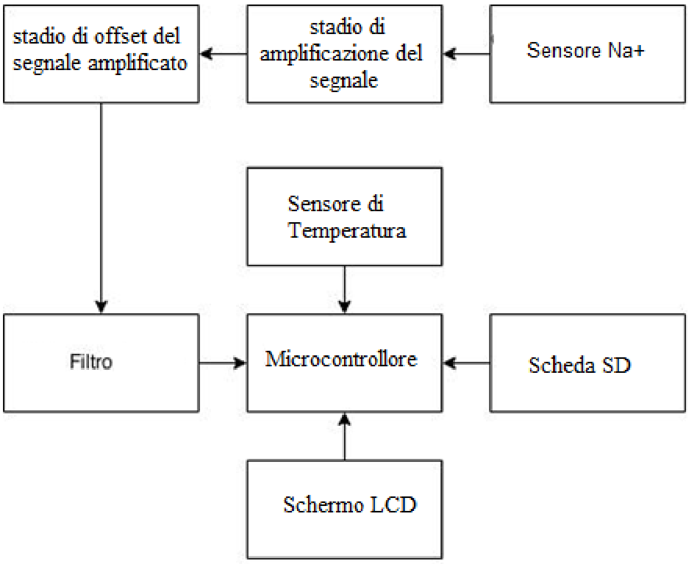


Figure 45. Block diagram of the device.

Statistical analyses were carried out using RStudio (version: 0.98.50), and in particular the ROCR (Sing et al., 2005) and pROC libraries (Robin et al., 2011). The values of the different traits measured in the milk are presented as arithmetic mean values and standard deviation. In addition, the correlations between the parameters were calculated.The values of the correlation coefficients for the variables under examination are showed in table 26. Over all possible combinations of parameters, the highest coefficients were found for Na+ with lactose (-0.817). The correlation between SCC and Na+ (r=0.727The number of somatic cells of the entire sample had a significant positive stable correlation with the ions Na + constant during all the lactation (table 27). A linear relathionships between SCC and Na+ were calculated for some samples to highlight the relationship between SCC and Na+ (figure 46 – 62).

Table 26. Correlation coefficients among milk variables in Sarda breed sheep milk (N=2000)

	Cas	Cell	Clor	Cond	Gras	K+	Latt	Lncell	Lnna	NA+	pH	Prot	UREA
Cas		0.25*	0.128*	-0.015	0.433*	-0.226*	-0.328*	0.349*	0.402*	0.342*	0.308*	0.989*	-0.078
Cell			0.486*	0.437*	0.145*	-0.293*	-0.51*	0.732*	0.61*	0.631*	0.639*	0.313*	-0.238*
Clor				0.963*	0.175*	-0.209*	-0.917*	0.565*	0.752*	0.804*	0.408*	0.22*	-0.243*
Cond					-0.075	-0.128*	-0.786*	0.502*	0.672*	0.729*	0.383*	0.091	-0.267*
Gras						-0.247*	-0.425*	0.232*	0.291*	0.252*	0.104*	0.377*	-0.088
K+							0.306*	-0.353*	-0.531*	-0.566*	-0.355*	-0.223*	0.087
Latt								-0.577*	-0.766*	-0.817*	-0.387*	-0.395*	0.112*
Lncell									0.775*	0.727*	0.575*	0.407*	-0.258*
Lnna										0.951*	0.525*	0.463*	-0.244*
NA+											0.609*	0.413*	-0.242*
pH												0.361*	-0.417*
Prot													-0.083
UREA													

<0.0001

Table 27. Correlation coefficents between SCC and Na+ from March to June

		March	April	May	June
SCC	**March**	**0.705***			
	April		**0.722***		
	May			**0.726***	
	June				**0.734***

Figure 46. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

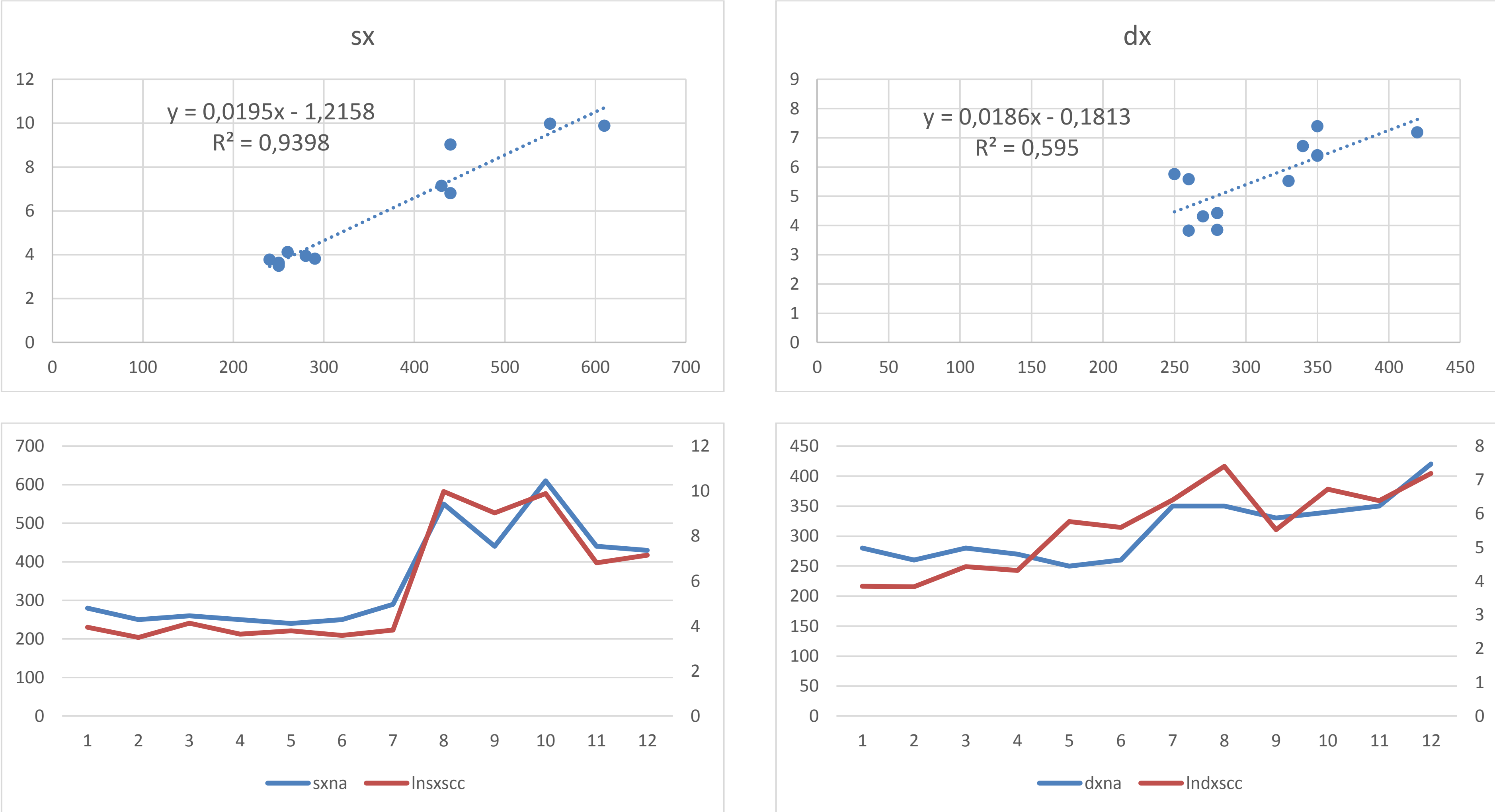


Figure 47. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

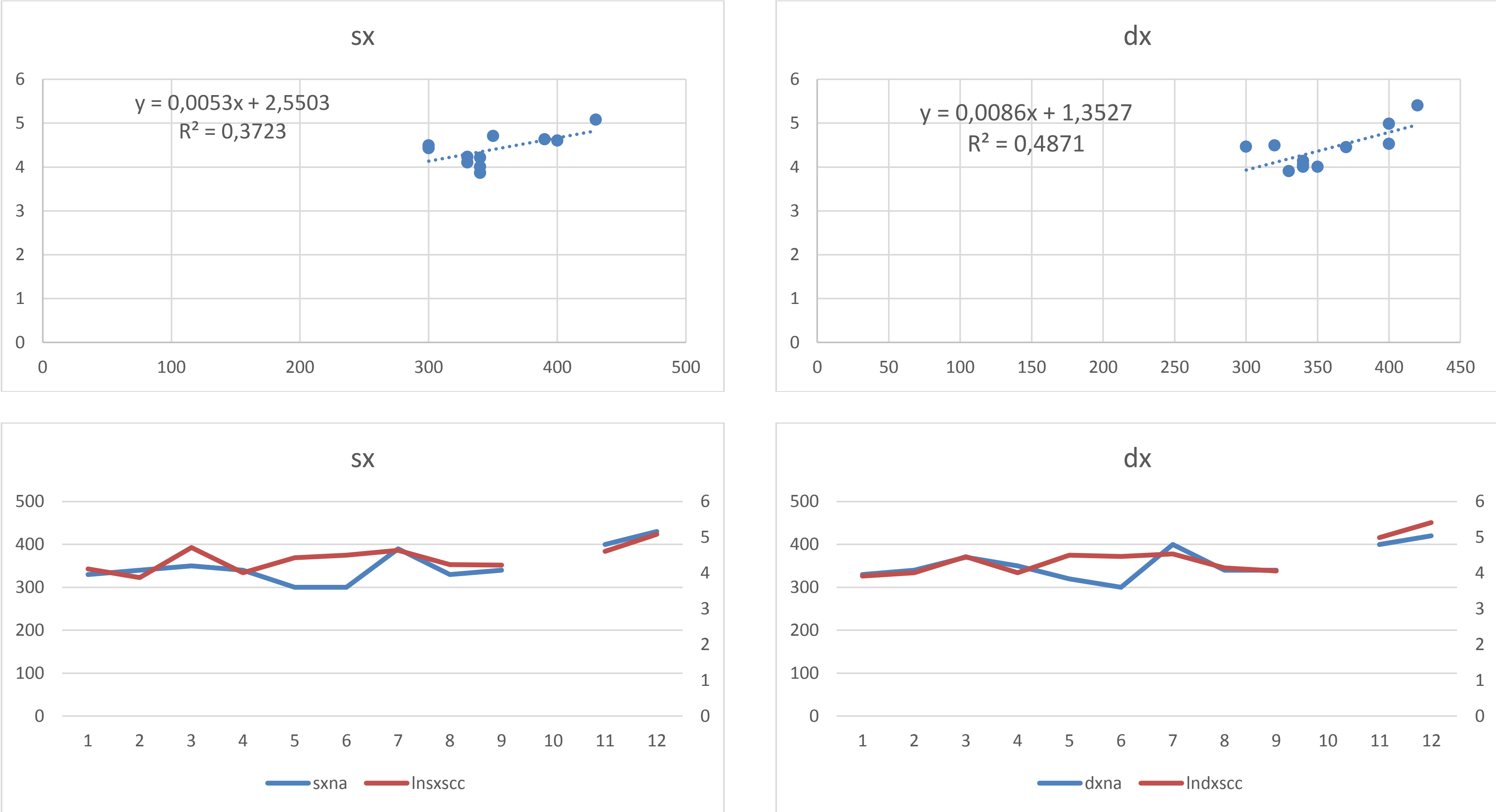


Figure 48. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

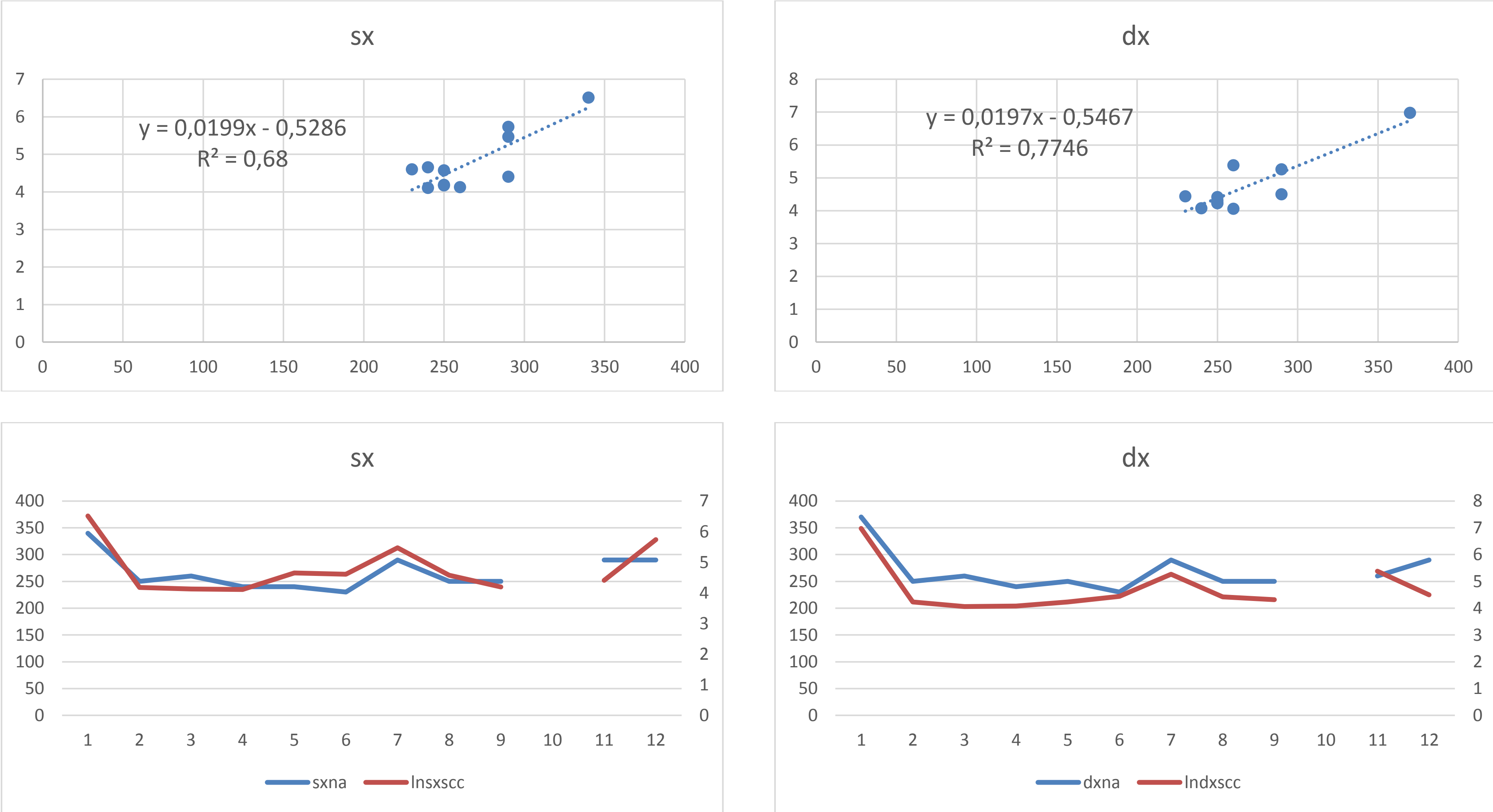


Figure 49. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

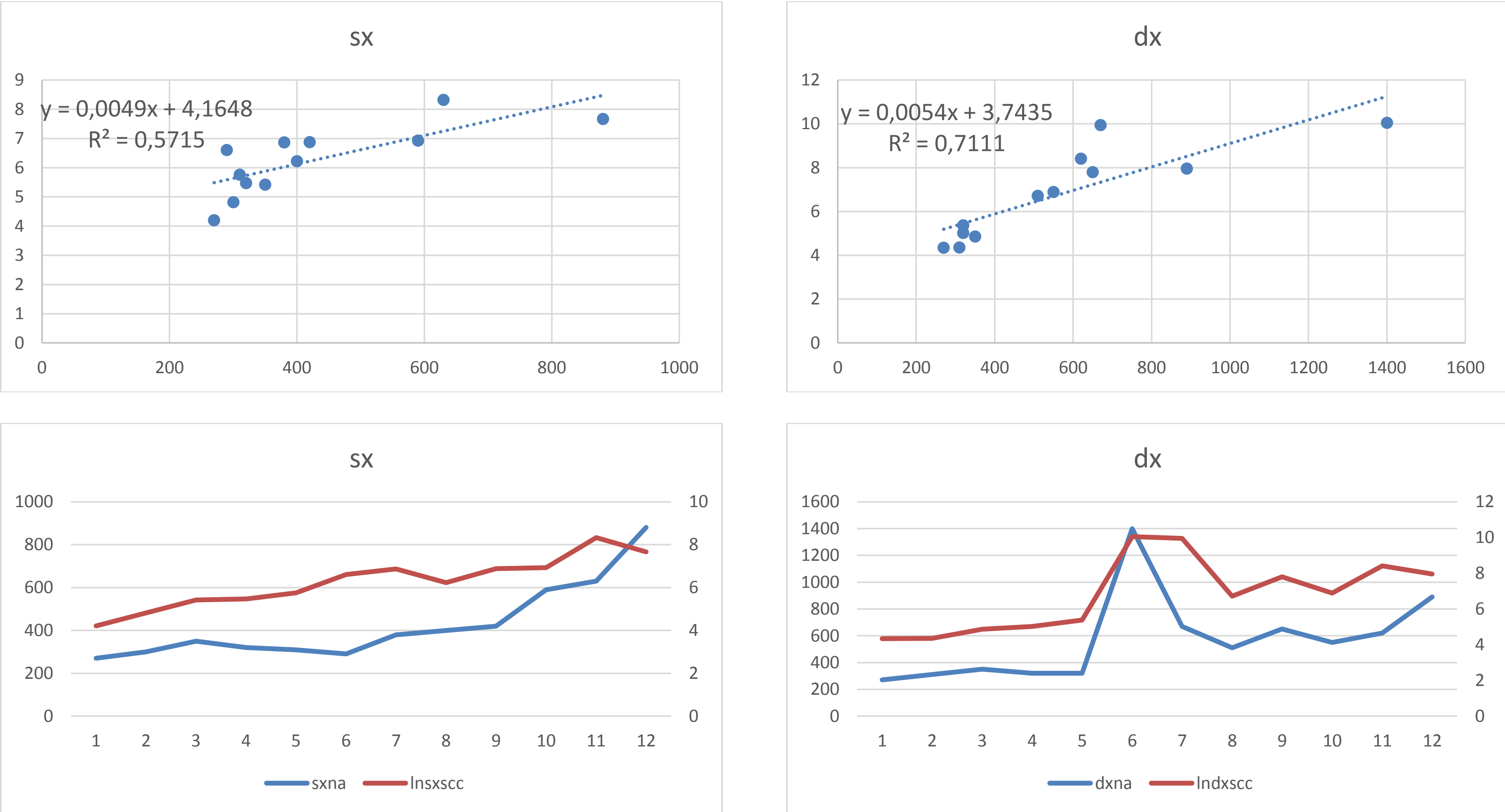


Figure 50. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

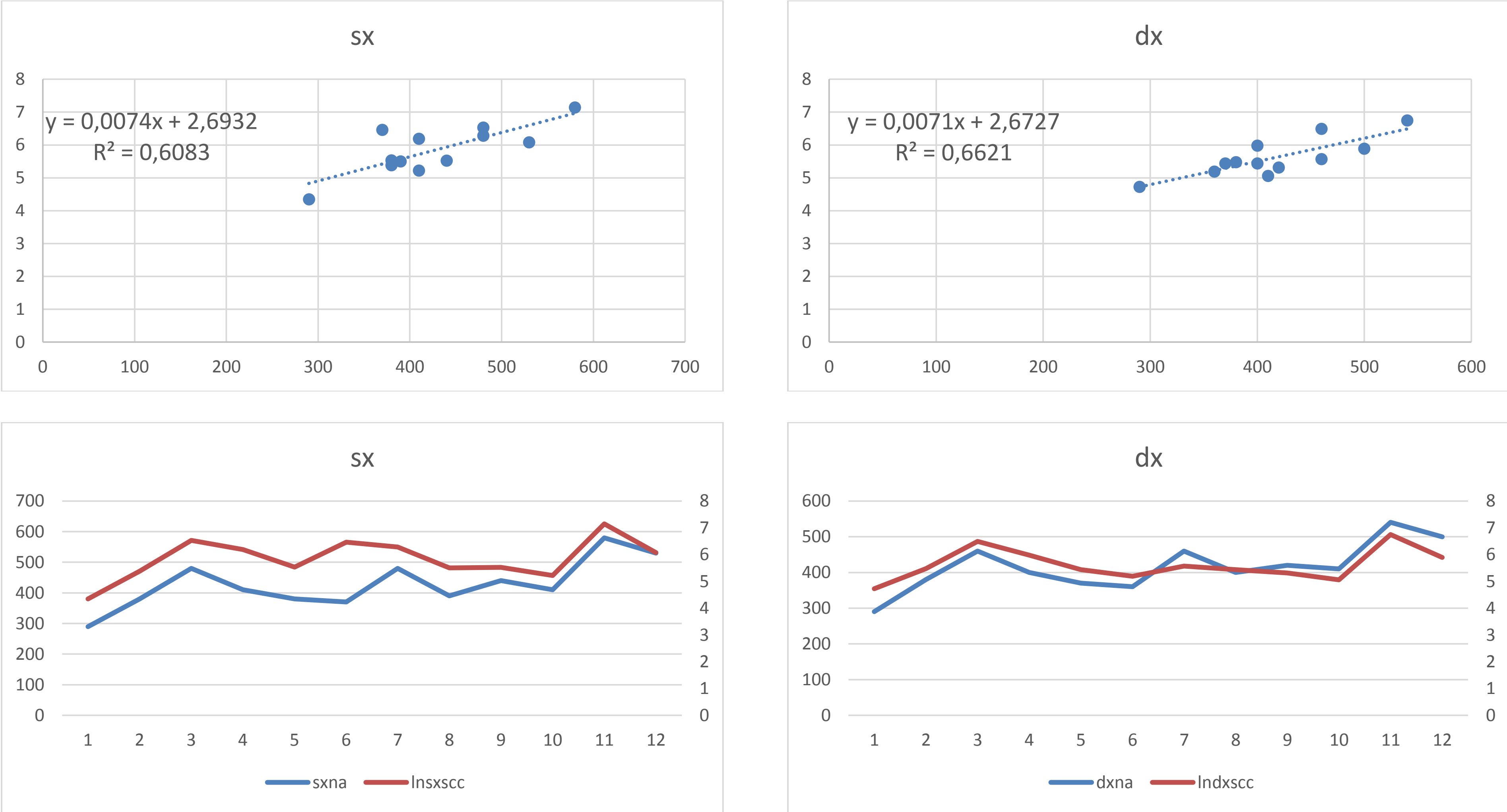


Figure 51. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

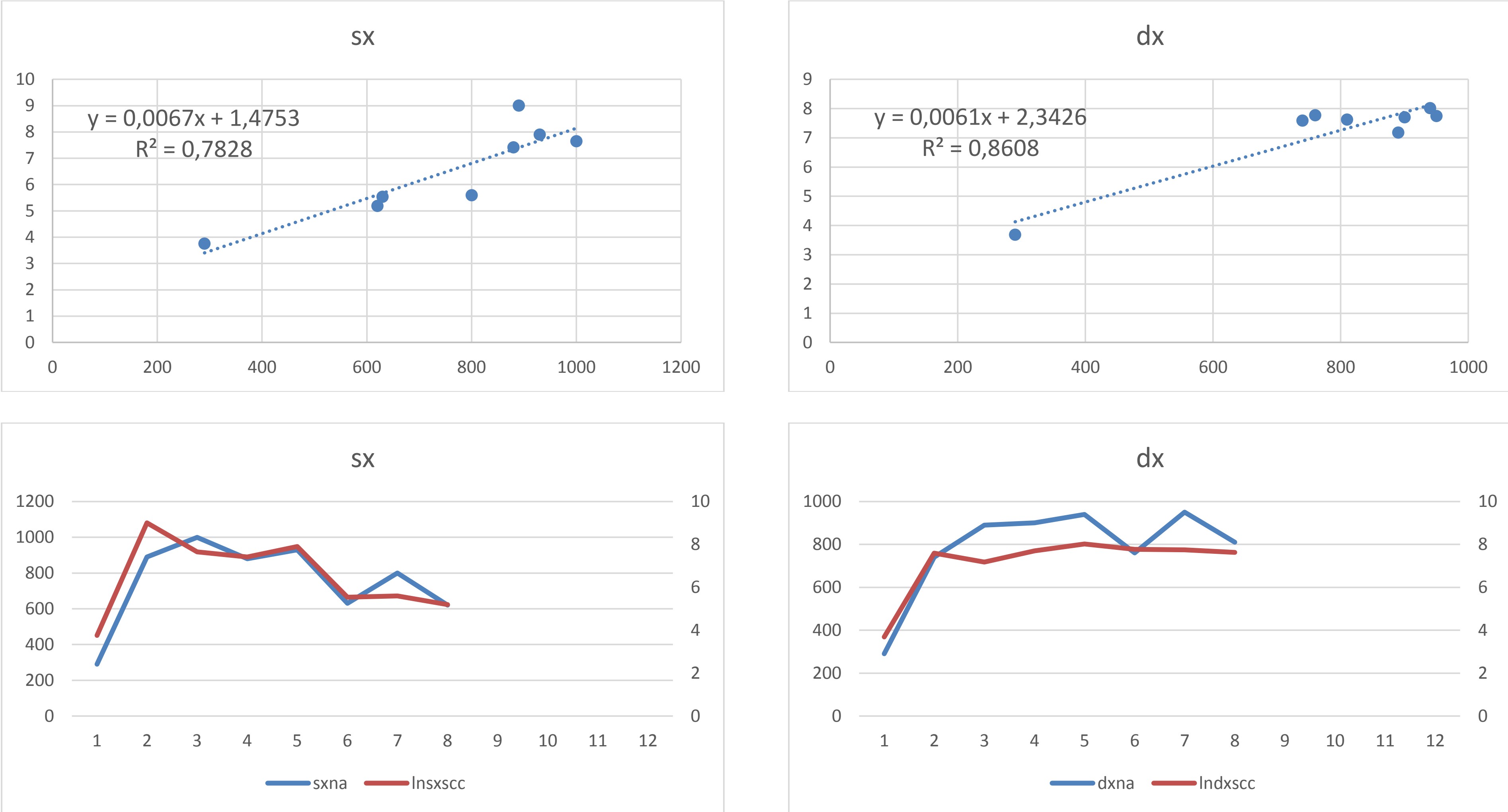


Figure 52. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

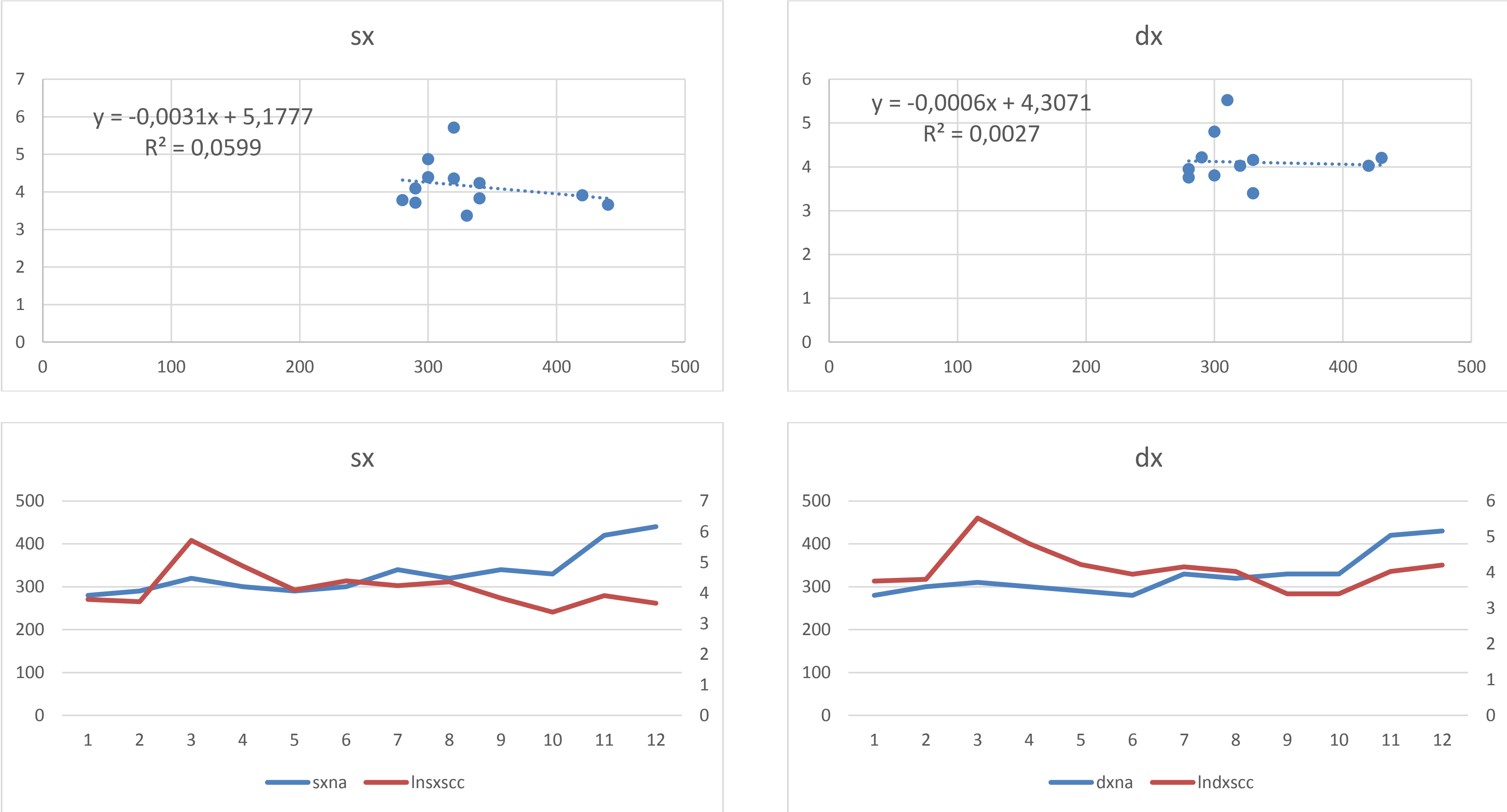


Figure 53. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

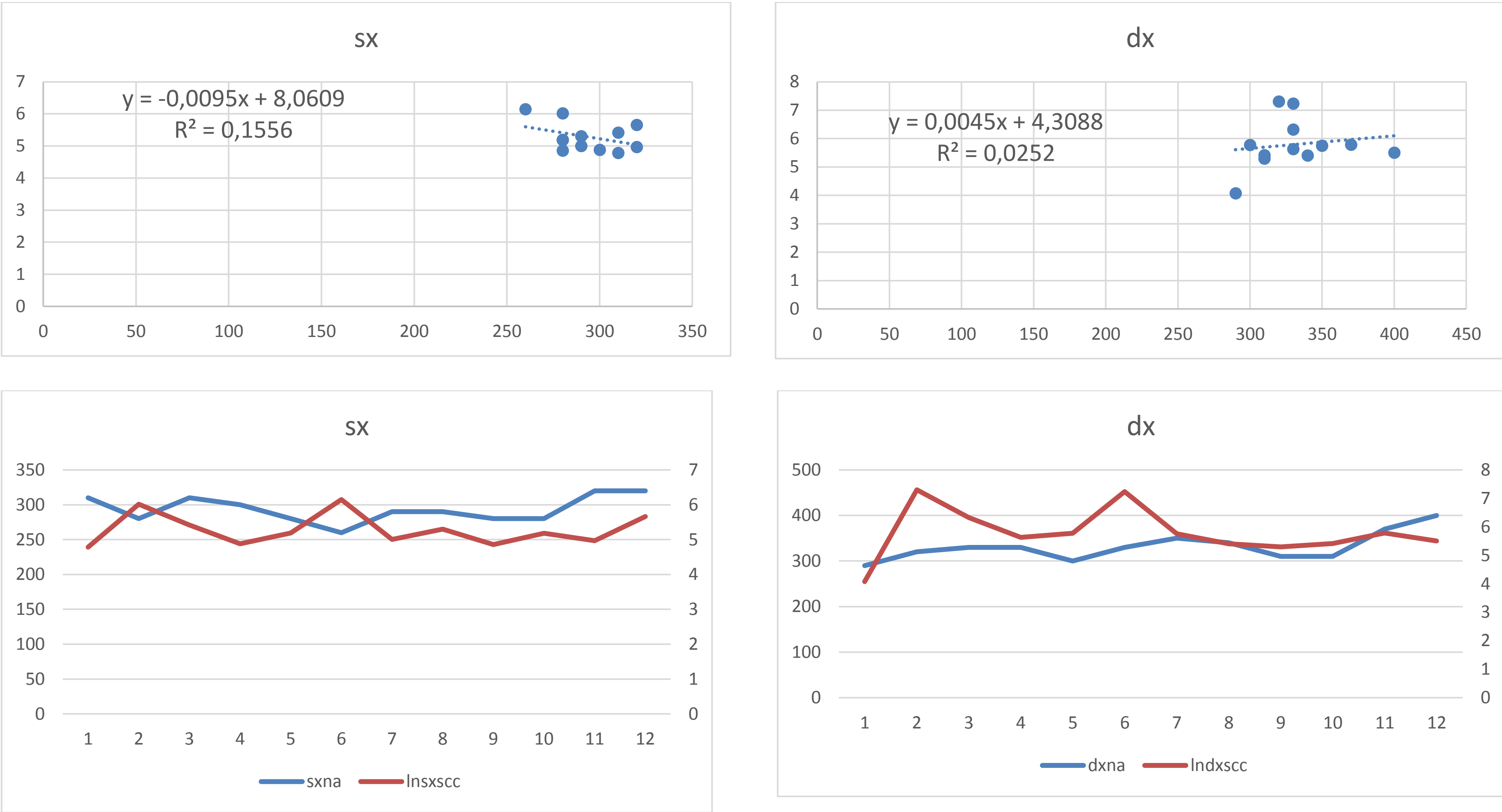


Figure 54. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

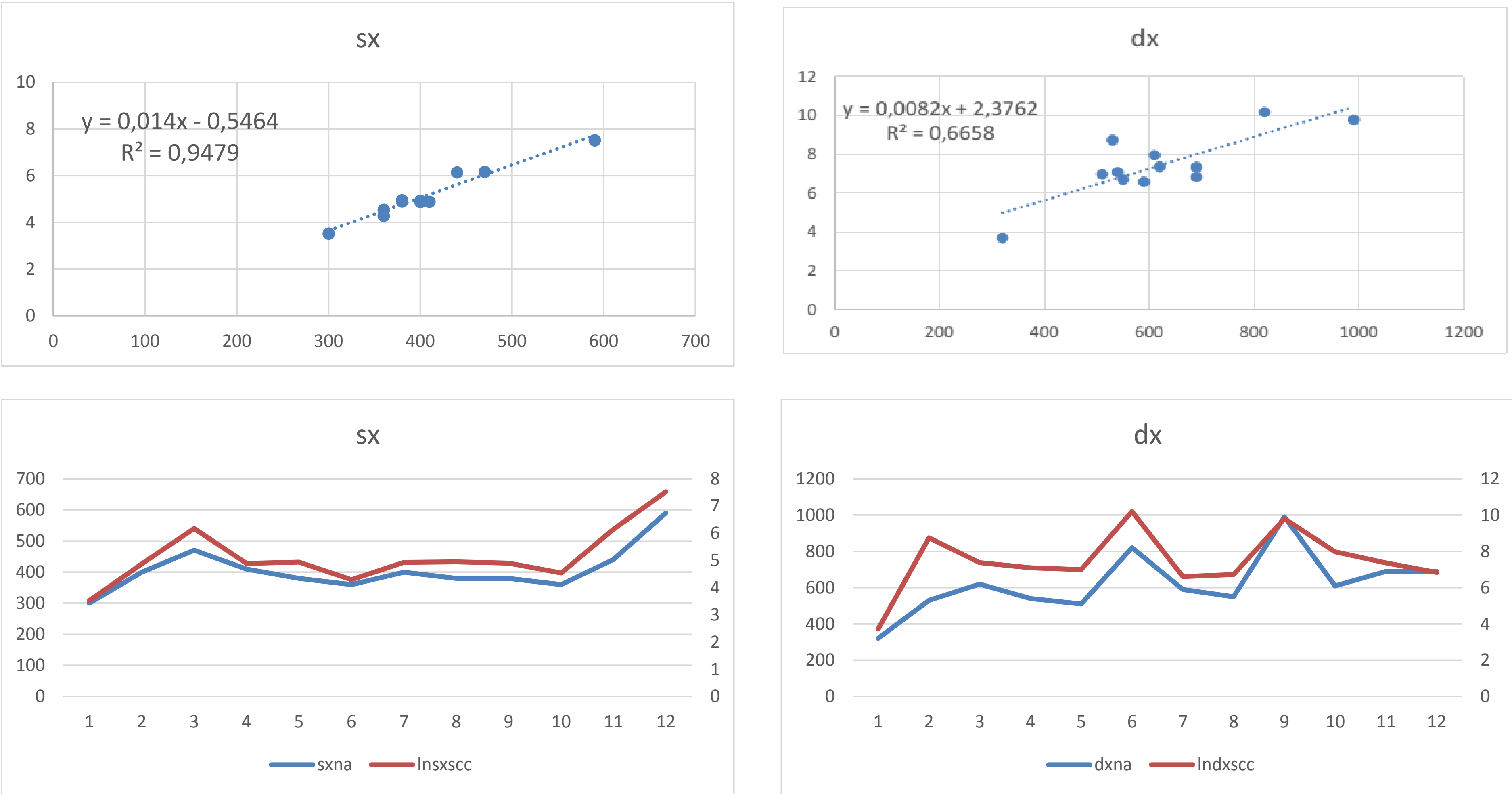


Figure 55. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

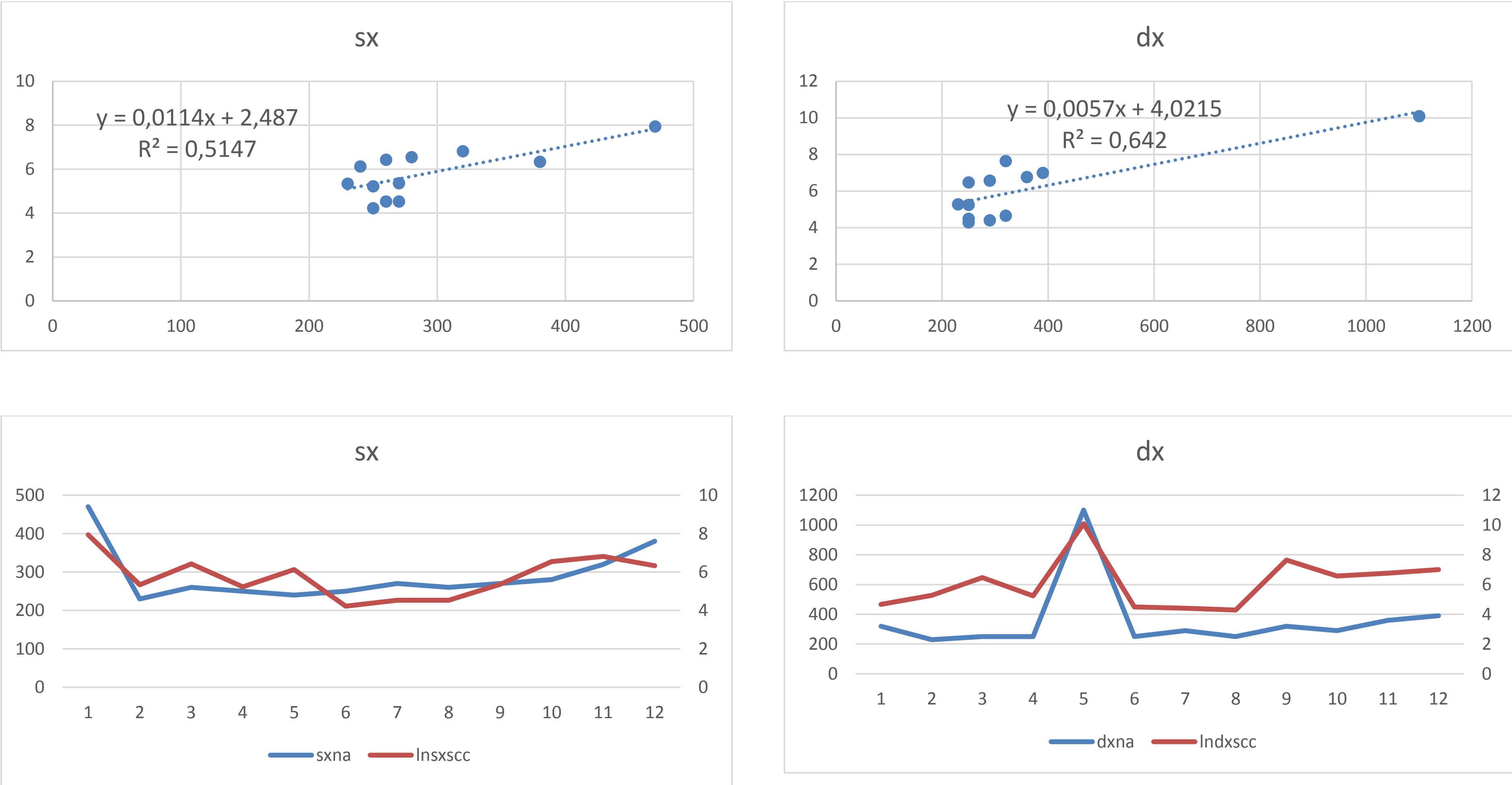


Figure 56. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

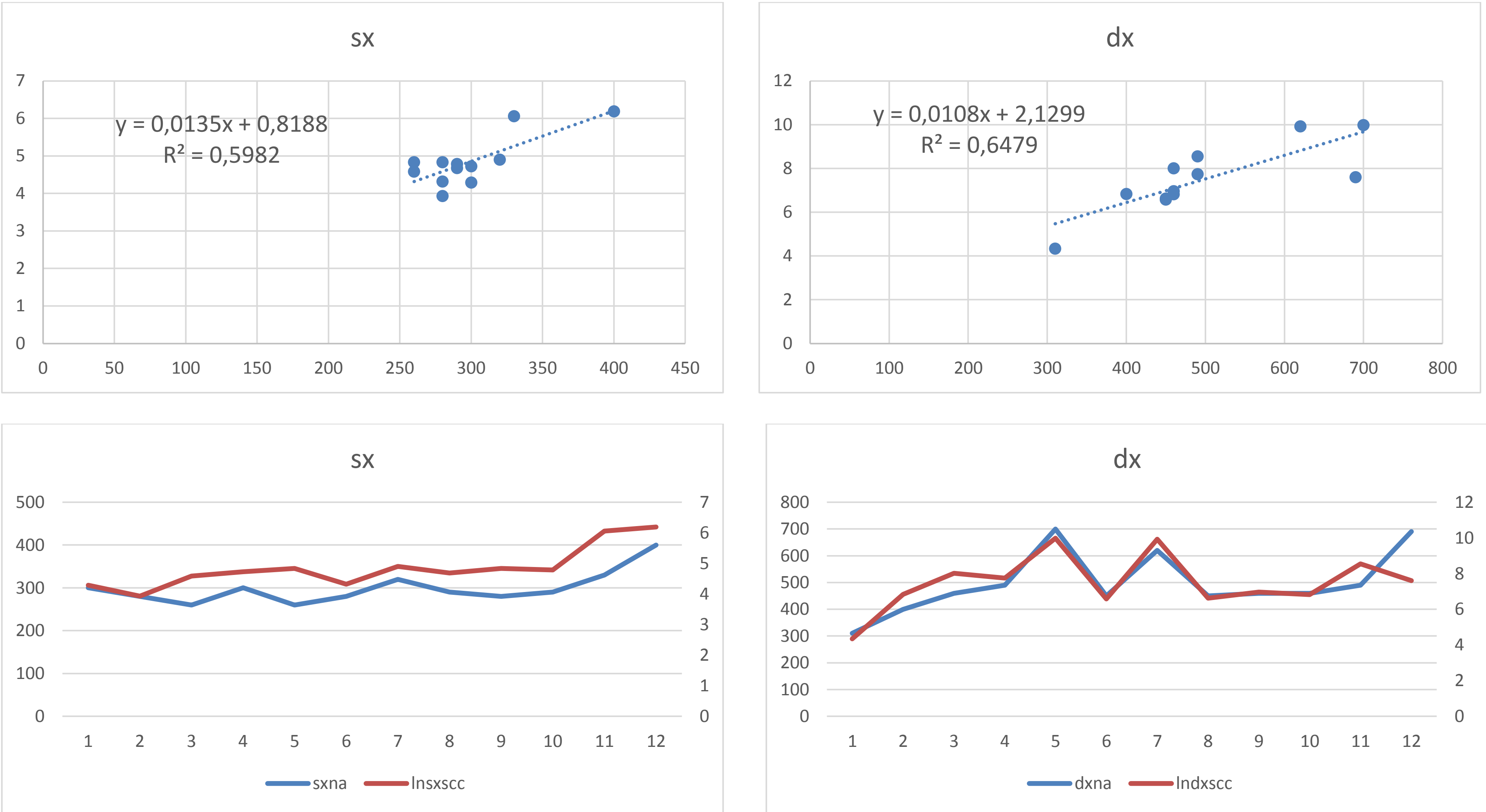


Figure 57. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

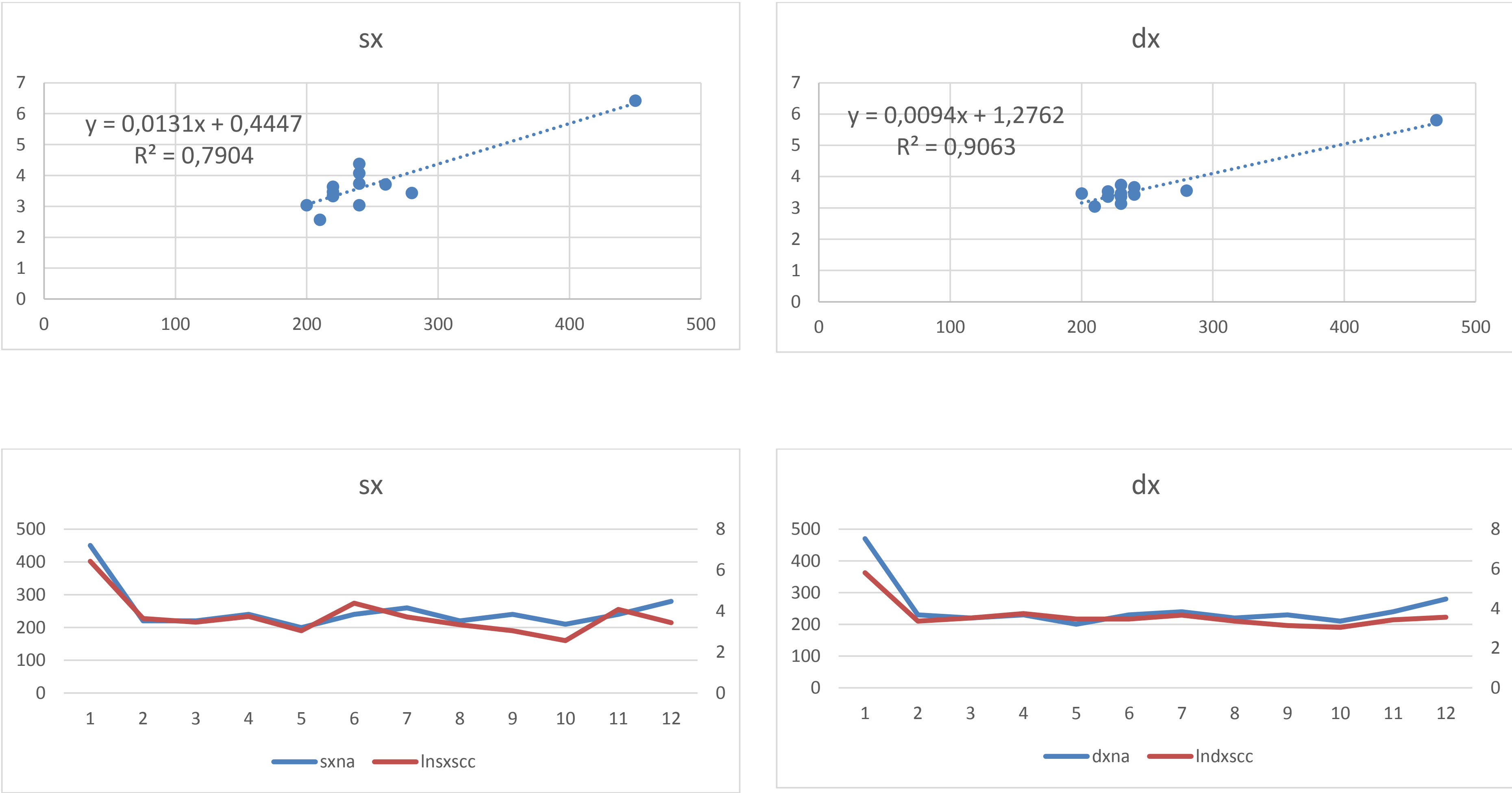
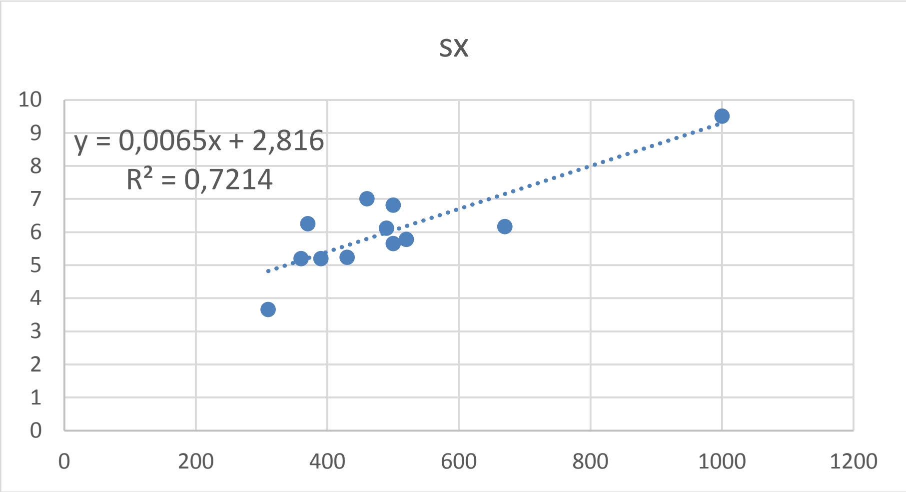
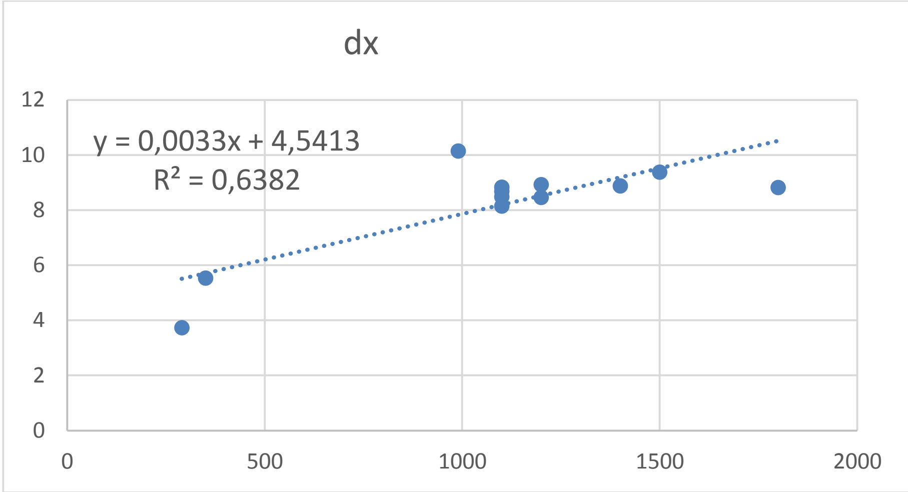
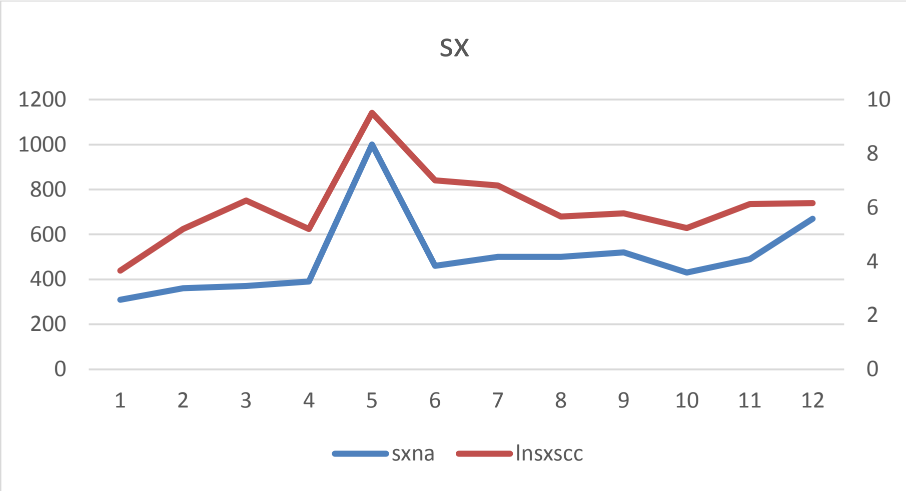


Figure 58. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.







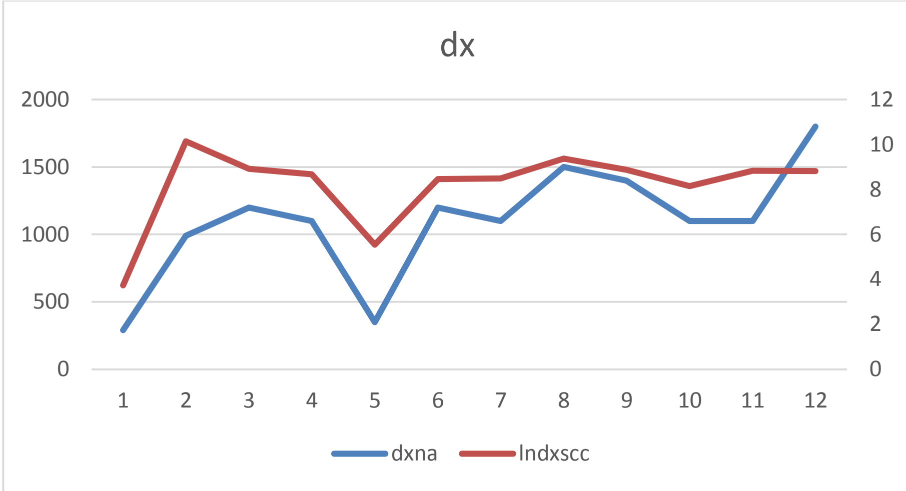


Figure 59. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

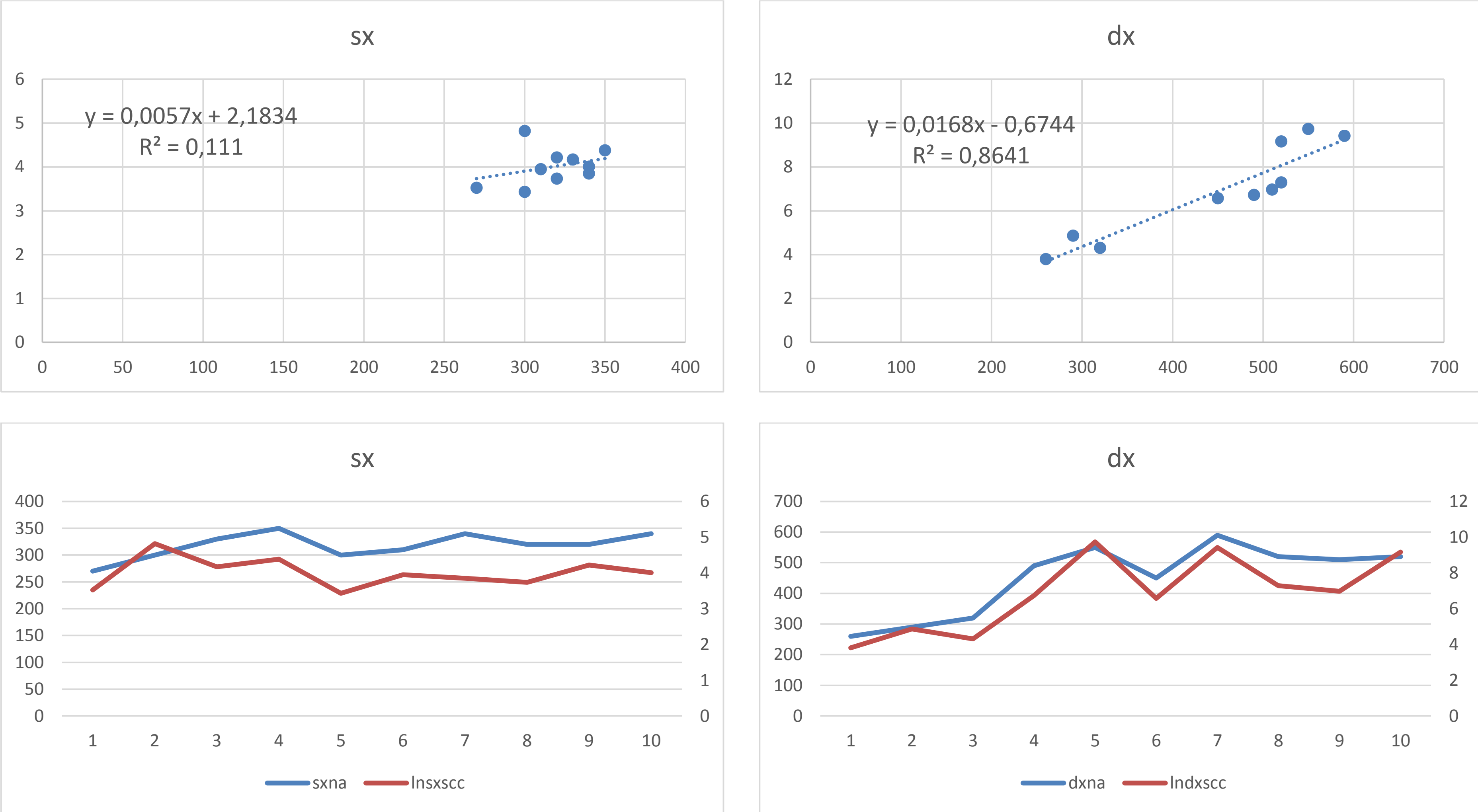
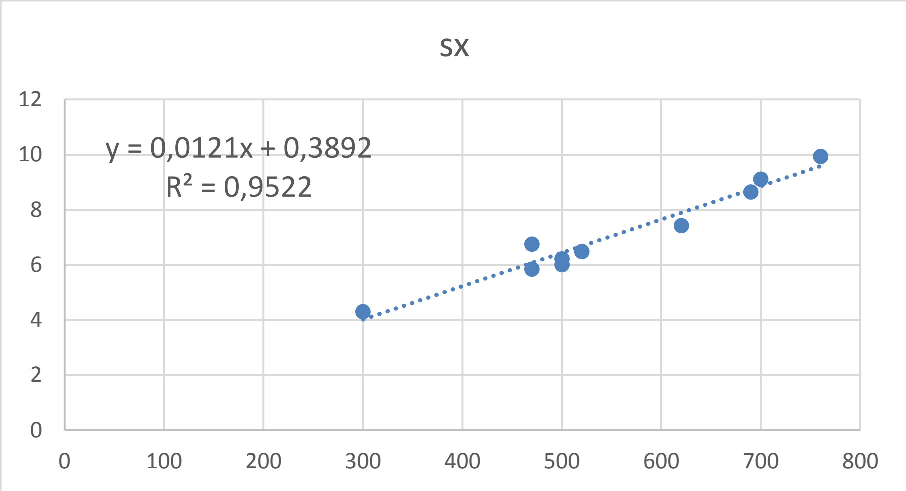
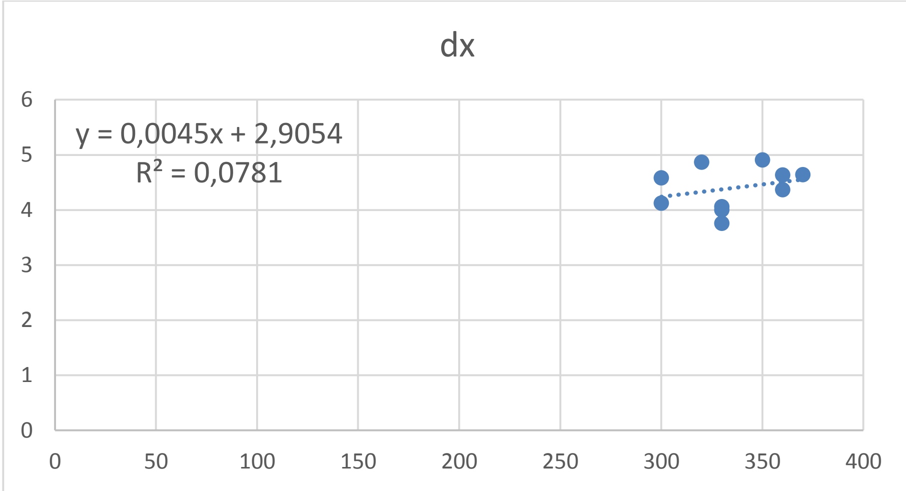
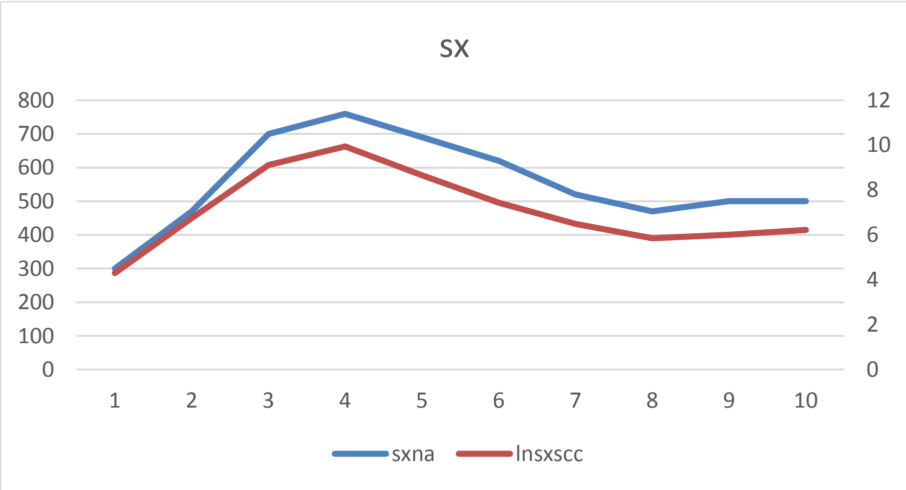


Figure 60. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database.

Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.







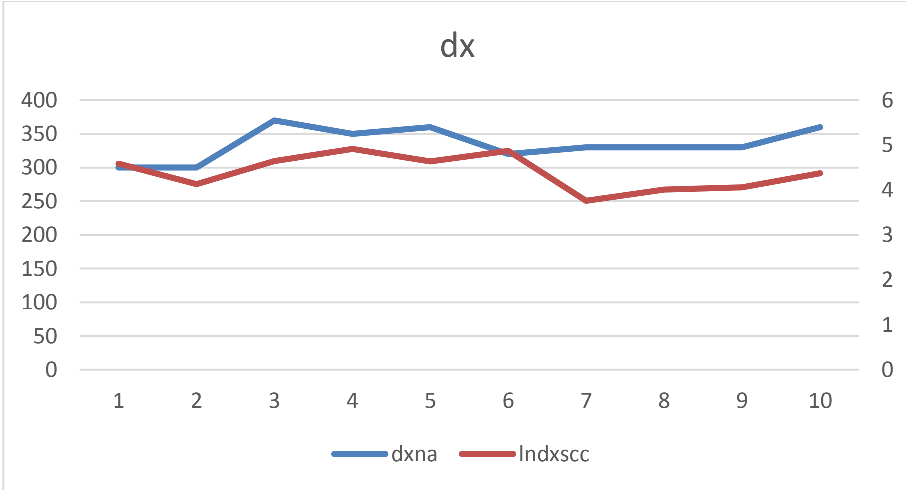


Figure 61. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.

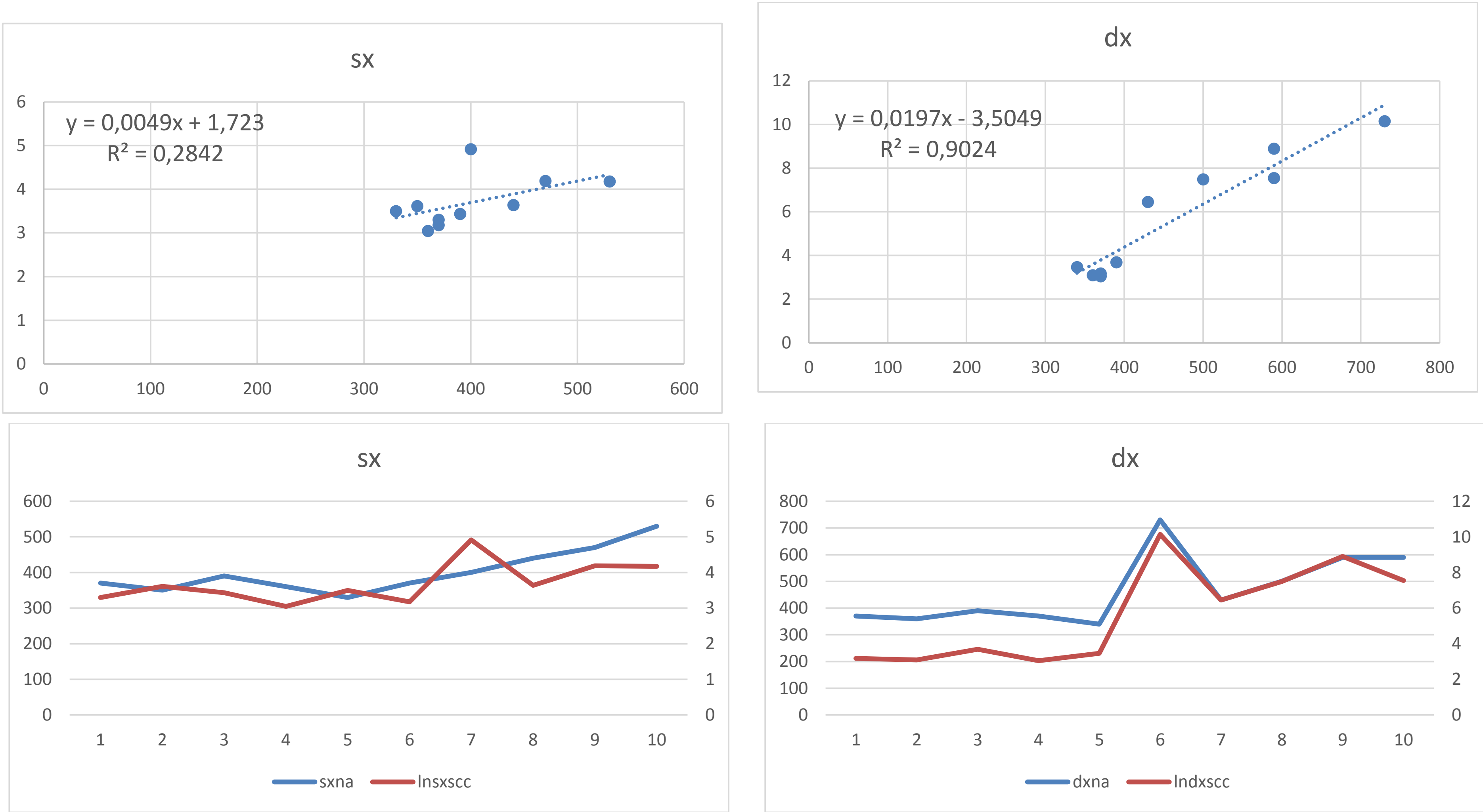
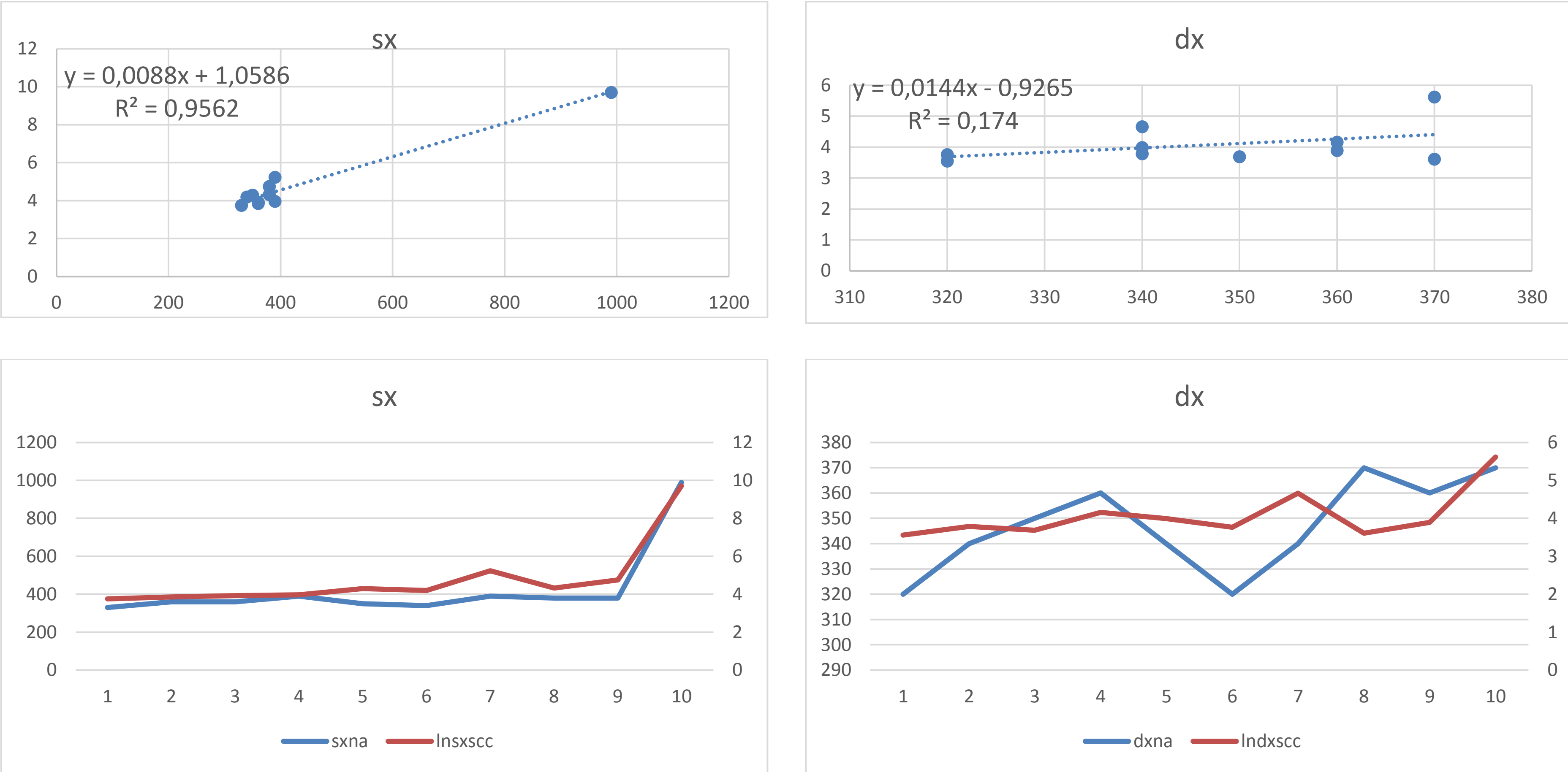
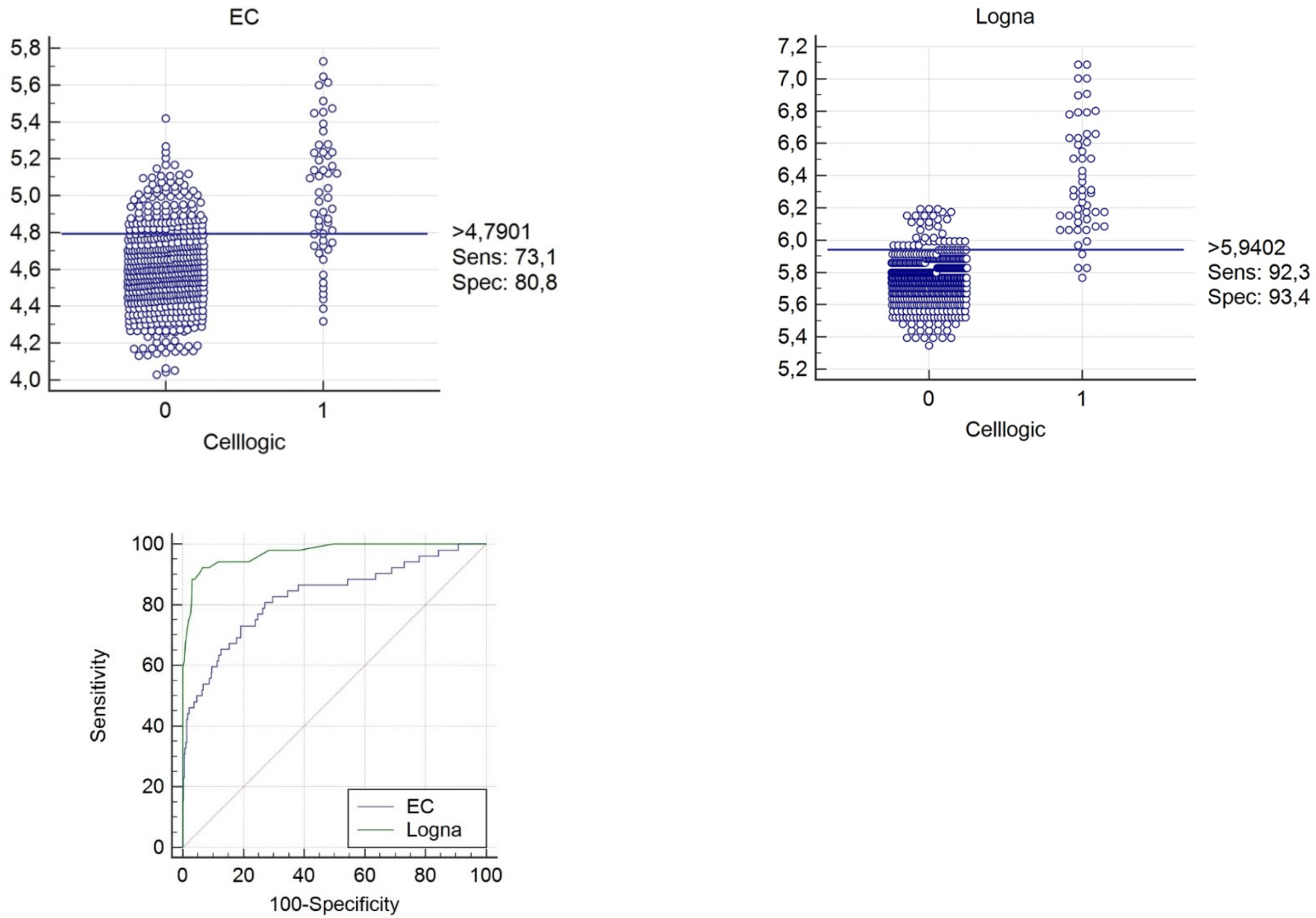


Figure 62. Upper, a linear regression calculation between Ln SCC and Na + ions for each emimammealla of a random sample from the database. Down the weekly trend, from March to July, the Ln of the SCC and the Na + ions for each emimammealla referring respectively to the figure above.



The ROC curves were elaborated from the conductivity and Na+ data which corresponded to the value of the cells ($700x10^3$), divided up as in Table 3. The AUC, which measured the diagnostic accuracy of the test, was 0.804 (P<0.0001) for EC and 0.95 (P<0.0001) for Na+. This indicated that the test was moderately accurate for EC and was very good for Na+ (figure 63).

Figure 63. Upper Dot plots, that is statistical chart consisting of data points plotted on a fairly simple scale, show difference between EC and NA+ ions. Down Receiver operating characteristic (ROC) curve confronts between sNa+ and EC (based on SCC equal to 700 x 10^3)



5.5 Conclusions

Somatic cell count (SCC) represents a marker to monitor the udder health in dairy ruminants. In a situation of non-specific illness, such as infections by microorganisms, shocks and/or injuries to the breast tissue, improper milking and environmental stress, the immune system sends in the breast the white blood cells and, consequently, milk presents an increase in SCC. The prototype developed incorporates two containers which receives milk samples taken from each half udder. The mathematical model, a linear relationship, loaded into the microcontroller by a firmware written in C / C ++, analyze the data and gives back the estimate of SCC level. The portable device can allows farmers to monitor the ewes health status by periodically comparing the SCC of each half udder.

The study showed that measuring the Na^+ is a useful way for identifying sheep with probable sub-clinical mastitis and thus reducing the costs of cyto-bacteriological analyses of the individual milk samples, the correlation between Na^+ and SCC corresponded to $R2 = 0.73$ ($P < 0.01$).

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Introduction / Chapter 1

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